UNITED STATES AIR FORCE

GRADUATE STUDENT RESEARCH PROGRAM

1989

PROGRAM TECHNICAL REPORT

UNIVERSAL ENERGY SYSTEMS, INC.

VOLUME 3 OF 3

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AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
BOLLING AIR FORCE BASE
WASHINGTON, DC

DECEMBER 1989

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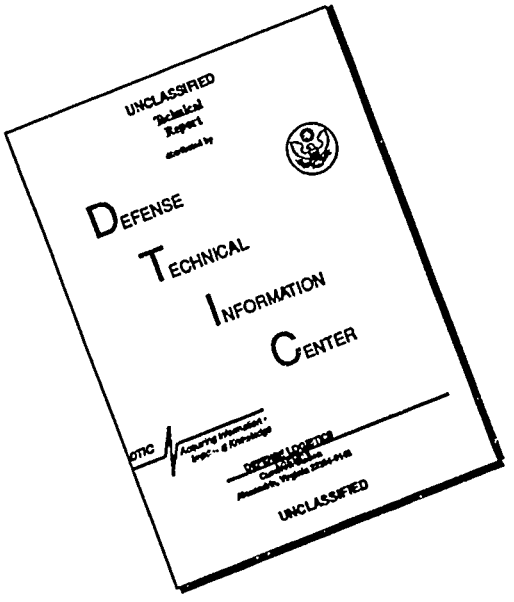

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PREFACE

The United States Air Force Graduate Student Resarch Program (USAF-GSRP) is conducted under the United Air Force Summer Faculty Research Program. The program provides funds for selected graduate students to work at an appropriate Air Force facility with a supervising professor who holds a concurrent Summer Faculty Research Program appointment or with a supervising Air Force Engineer/Scientist. This is accomplished by the students being selected on a nationally advertised competitive basis for a ten-week assignment during the summer intersession period to perfrom research at Air Force laboratories/centers. Each assignment is in a subject area and at an Air Force facility mutually agreed upon by the students and the Air Force. In addition to compensation, travel and cost of living allowances are also paid. The USAF-GSRP is sponsored by the Air Force Office of Scientific Research, Air Force Systems Command, United States Air Force, and is conducted by Universal Energy Systems, Inc.

The specific objectives of the 1989 USAF-GSRP are:

(1) To provide a productive means for the graduate students to participate in research at Air Force Laboratories/Centers;

(2) To stimulate continuing professional association among the graduate students and their professional peers in the Air Force;

(3) To further the research objectives of the United States Air Force;

(4) To enhance the research productivity and capabilities of the graduate students especially as these relate to Air Force technical interests.

During the summer of 1989, 102-graduate students participated. These researchers were assigned to 23 USAF laboratories/centers across the country. This three volume document is a compilation of the final reports written by the assigned students members about their summer research efforts.

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UNITED STATES AIR FORCE

GRADUATE STUDENT RESEARCH PROGRAM

1989

PROGRAM TECHNICAL REPORT

UNIVERSAL ENERGY SYSTEMS, INC.

VOLUME III of III

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Submitted to

Air Force Office of Scientific Research

Bolling Air Force Base

Washington, DC

December 1989

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PREFACE

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(4) To enhance the research productivity and capabilities of the graduate students especially as these relate to Air Force technical interests.

During the summer of 1989, 102-graduate students participated. These researchers were assigned to 23 USAF laboratories/centers across the country. This three volume document is a compilation of the final reports written by the assigned students members about their summer research efforts.

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FINAL REPORT

RESEARCH INTO SEMEN ANALYSIS AS A SENSITIVE INDICATOR OF NEUROTOXICITY

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Research Into Semen Analysis as a Sensitive Indicator of Neurotoxicity

by

R.M. Barbaro and J.R. Cooper

ABSTRACT

Air Force, Navy, and Marine Corps personnel are routinely exposed to a variety of potentially neurotoxic substances. The sooner adverse consequences resulting from exposure to these agents are detected, the sooner measures can be taken to prevent irreparable damage. The purpose of this study was to determine if analysis of semen can be used to indicate neurological damage caused by exposure to volatile chemicals. Three projects were initiated to prepare for this investigation. First, ceramic glass reservoir systems were fabricated using high density (2.628 ± 0.046 g/cm^3) ALCAP ceramics. This system provided for the slow release of the test chemical, 1,1,1-trichloroethane in the animal. Second, a system to detect 1,1,1-TCE using a GC-FID rather than a GC-ECD was investigated. Finally, the computerized semen analyser was set up and a familiarity with the system was gained.

ACKNOWLEDGMENTS

I would like to thank the Air Force Systems Command and Air Force Office of Scientific Research for their sponsorship of the program, as well as Universal Energy Systems for their support. Special thanks also to Dr. James Cooper for his guidance and support throughout the program.

For all the hours spent at the Naval Medical Research Institute Toxicolgy Detachment learning and using the gas chromatograph, I would like to thank Colonel Macys and all the friendly people there. I would especially like to thank Jose Rivera for patiently spending much of his time teaching me how to run the GC.

Acknowledgements are especially due to Dr. P.K. Bajpai for letting us use his laboratories for fabricating the CGRS and for giving guidance and moral support during the tenure of this project. I also appreciate the time, expertice, and technical assistance that Hamed Benghuzzi, Hisham Arar, Anne Marie Barbaro, and especially Brian Granite gave me. Their initiative, support, and friendship will never be forgotten.

I. INTRODUCTION

Naval, Air Force, Marine Corps and other military personnel are exposed to a variety of chemical agents during their duties. These chemicals could possibly produce neurological effects and thus reduce health and impair performance. A simple method to screen potential toxicants is needed to reduce these risks. During this research period we wanted to determine whether semen analysis could be used as a sensitive indicator of neurotoxicity.

Recent interest in the study of infertility has lead to a much greater understanding of gamete function and its relation to the central nervous system. This interaction occurs primarily through the gonadotropic hormones LH and FSH. LH stimulates the Leydig cells of the testis to produce testosterone which is needed for growth and development of accessory sex organs. FSH targets sertoli cells where the differentiation of sperm occurs. This system is regulated by a negative feedback mechanism associated with the hypothalamus. It is therefore possible that agents which affect the central nervous system may indirectly produce effects on sperm production by altering gonadotropic hormone levels (7, 11).

Sperm development is a highly complex process. Environmental agents are capable of altering this process and resulting in the production of damaged or abnormal sperm. Sperm development is normally protected from xenobiotic agents in the blood by a biological barrier known as the blood-testis barrier. This barrier is similar in function to the blood-brain barrier. Both barriers function in a similar fashion by

screening chemicals according to molecular size, polarity, and lipid solubility. Due to the similarity between the barriers, it is hypothesized that agents capable of crossing the blood-testis barrier and affect sperm production may also be able to cross the blood-brain barrier and affect the functioning of the central nervous system. A reproductive toxicant may therefore affect sperm production either indirectly by altering neurologic functioning and subsequently blood endocrine levels or directly by influencing testicular function. Either effect may give an indication of the potential for the agent to cross the blood-brain barrier (7, 11).

Computerized videomicrography has been used to detect and quantify various sperm parameters including concentration, motility, and morphology. This method of computer assisted semen analysis provides for a faster and more accurate assessment of semen quality than can be obtained by subjective visual assessment. The effect of toxic agents on the sperm can be determined by noting the concentration, percent motility, and swimming speed of sperm in the sample. The most sensitive indicators being sperm motility and velocity (15).

1,1,1-Trichloroethane (1,1,1-TCE), a volatile chlorinated hydrocarbon, is an organic solvent widely used as a degreasing agent which can also be found in commercial aerosol and adhesive products (12). It is a non-flammable, colorless liquid with a boiling point of 74 °C and has shown to be a central nervous system depressant (3,4,9,10,12). Absorption through the skin or inhalation of 1,1,1-TCE may cause dizziness, uncoordination, and eventual death due to respiratory failure (12).

In the past, exposure studies involving volatile hydrocarbons such as 1,1,1-TCE had to be conducted utilizing inhalation, intraperitoneal injection, or oral dosage methods of administration making continuous low level exposures to these agents difficult. Recently an implantable ceramic glass reservoir system has been designed to deliver volatile chemicals at sustained rates (4,5,6).

The ceramic glass reservoir system (CGRS) consists of a 1 cm x 4 cm cylindrical glass reservoir attached at one end to a porous alumino-calcium-phosphorous oxide (ALCAP) ceramic capsule and sealed at the other end with a rubber septum. This system allows for a sustained release of the volatile chemical from the reservoir through the ALCAP ceramic to the subcutaneous tissue of the animal for several hours or days. To date, ALCAP ceramics have been used to deliver enzymes, dyes, phenolics, polypeptides, proteins and steroids in a sustained manner both in vitro and in vivo (1,2,13).

The CGRS has been found to be biocompatable and causes minimal trauma and no restriction to the normal activity of the animal (4,5). This controlled release device eliminates the pulsatile delivery of the substance associated with the injection route and allows for attainment of steady blood concentrations which are difficult to achieve with other methods of 1,1,1-TCE administration including inhalation exposure. This means that higher blood levels of 1,1,1-TCE can be achieved using lower amounts of the chemical (4,5,8).

II. Objectives of the Research Effort

The purpose of this study was to set up a system that could determine whether semen analysis could be used to indicate neurological damage caused by exposure to volatile chemicals. Although behavioral, hematologic, and pathologic methods have been developed to indicate neurotoxicity, semen analysis could possibly provide an earlier and more sensitive indication of tissue damage.

Work under the 1989 Graduate Student Research Program required me to set up and evaluate various aspects of the project. One task involved fabrication of the ceramic glass reservoir systems. This task also involved characterization of the system and preliminary studies both in vitro and in vivo with the CGRS.

A second task dealt with detection of the 1,1,1-TCE in the blood. Usually a gas chromatograph fitted with an electron capture detector is used for this purpose, but the only GC available for our use was equipped with a flame ionization detector. Thus, a system had to be designed to identify 1,1,1-TCE using the GC-FID.

Finally, the semen analyzer had to be assembled and validated. The CellSoft (CRYO Resources, Ltd., New York) computerized semen analyzer consists of several parts including an Olympus microscope equipped with a "Fryer" (Fryer Co. Inc., Carpentersville, IL) stage warmer, a NEC computer with Epson LX-800 printer, and

Panasonic Omnivision VHS and TV monitors. After assembly of these parts work on understanding and implementing the CellSoft program proceeded.

III. The Ceramic Glass Reservoir System

The ceramic glass reservoir system was designed by Hollenbach and Bajpai to provide a delivery system for volatile materials that is easy to use and does not hinder the normal activity of the test animal (4,5). The CGRS they developed consists of a glass reservoir attached to a porous ALCAP ceramic. These biocompatable systems can be implanted subcutaneously in the animal and then injected with the volatile material. A sustained rate of release of the chemical lasts for a period of days and, when empty, the reservoir may be refilled with another injection.

Alumino-calcium-phosphorous oxide (ALCAP) ceramics were fabricated by mixing aluminum oxide, calcium oxide, and phosphorous pentoxide (50:34:16 by weight). This mixture was then calcined in a high temperature furnace (Thermolyne, Inc., Deubuque, IA) at 1350 °C for 12 hours. It was then ground in a jar mill and sized with a 400 mesh screen (Fisher Scientific Co.), so particles less than 38 microns in diameter were collected. The cylindrical shape of the capsule was made by compressing 1 gram of ALCAP mixed with 0.025 grams polyvinyl alcohol in a 5/16" die set with a French Cell Press (American Instruments Co., Silver Spring, MD). These "green-shape" capsules were then sintered at 1450 °C for 36 hours to increase mechanical strength. Wheaton autosampler vials (2 ml) were modified by the University of Dayton Glassblowing

Laboratory by attaching a 1 cm x 4 mm glass tube with an opening of 1 mm to one end. The 1 mm opening was used since Hollenbach had found that rate of release of the 1,1,1-TCE from the CGRS was constant up to a 2 mm opening, beyond which the release was much faster (4). The other end of the vial was closed with a crimp top fitted with a rubber and teflon septum. These reservoirs were then sealed to the ALCAP capsules with Silastic Medical Adhesive, Silicone Type A (Dow Corning).

High density ceramics with thicker walls were fabricated in an attempt to decrease the rate of release of the volatile material. A preliminary study was conducted to determine what effects density may have on the rate of release of 1,1,1-TCE from the CGRS. Capsules of low, medium, and high densities (1.853 $\pm$ 0.037, 2.073 $\pm$ 0.038, and 2.144 $\pm$ 0.02 g/cm^3 respectively) were fabricated and sealed to the glass reservoirs. These were then placed in vials containing 15 mls each of CS_2 and injected with 1 ml of high purity 1,1,1-trichloroethane (99.9% pure Aldrich Chemical Co., Milwaukee, WI). It was noted that the high density ceramic markedly decreased the release rate of 1,1,1-TCE. Slow release rate could possibly be due to the solvent that the 1,1,1-TCE was being released into and therefore may not be an accurate representation of delivery in vivo. The high density ceramic was chosen to be used in an in vivo study to determine its rate of release in the live animal.

IV. Blood Chemistry

Gas chromatography provides a means of separating complex mixtures into their

component parts. The volatilized material is injected into a column holding the stationary phase. Each component of the mixture is retained by the stationary phase for a finite period of time depending on its particular physical properties such as molecular weight and electrical charge. The retention times act as a fingerprint of the substance. This process can be used to identify and calculate concentrations of a desired component. In this study gas chromatography was employed to measure the concentration of 1,1,1-TCE in the blood of the experimental animal.

A GC-ECD is normally used to detect 1,1,1-TCE because it is specific for halogenated compounds. Unfortunately, the only GC available for use at the time of the study was equipped with a flame ionization detector (FID). The FID was incapable of detecting 1,1,1-TCE when the traditional hexane solvent was employed, so studies are currently in progress to identify a solvent system which will enable the quantification of 1,1,1-TCE utilizing the FID system.

V. Semen Analysis

The CellSoft (CRYO Resources, Ltd., New York) automated semen analyzer is able to track and record sperm motion parameters so that assessments of the semen quantity and quality can be made. CellSoft distinguishes sperm cells from backround on the basis of size, motion, and luminosity, or illumimation of the sperm head. Once the differentiation has been made, measurements such as percent motility, concentration, curvilinear velocity, linearity, amplitude of lateral head displacement (ALH), and

beat/cross frequency can be made. From these data an analysis of semen quality can be determined.

Prior to analyzing sampled sperm, critical measurement parameters must be established. They were: the number of frames to analyze = 20; number of frames/sec = 30; minimum sample for velocity = 3 frames; minimum sample for motility = 2 frames; maximum velocity = 300 um/sec; threshold velocity = 20 um/sec; cell size range from 20-125 pixels; pixel scale = 3.4 um/pixel; minimum velocity for ALH = 20 um/sec; minimum linearity for ALH = 3.5; minimum velocity for circular motion = 20 um/sec; and maximum radius for circular motion = 80 um. Considerable time was then spent on becoming familiar with the system.

At the end of the 10 week period a preliminary in vivo study was initiated by implanting six male rats with a CGRS. Each rat was anesthetized with an intraperitoneal injection of a mixture of 70 mg/kg Ketamine and 6 mg/kg xylazine then an area of skin on the back was shaved and disinfected. A centimeter incision was made in the skin and the CGRS was implanted subcutaneously. The incision was then closed with silk sutures and the animals were allowed to heal for one week before dosing.

After 1 week, each animal was injected with 1 ml of 1,1,1-TCE by inserting a hypodermic needle through the skin and rubber septum of the CGRS. The capsules of the CGRS had a density of 2.628 $\pm$ 0.046 g/cm^3 and were expected to deliver the 1,1,1-TCE at a sustained rate for 3 days before the reservoir w[illegible]ty.

VI. Recommendations

a. High density ALCAP ceramics were used in the ceramic glass reservoir system to obtain a slower release of 1,1,1-TCE. The size of the capsule can be increased by using more ALCAP powder (1.25 g) or larger dies. This will facilitate exchange by increasing the surface area of the ceramic. The glass reservoirs can accommodate approximately 1.5 ml of 1,1,1-TCE, and for greater amounts double implants could be employed.

b. Further work on the GC-FID is required to find suitable methodology for detecting 1,1,1-TCE. Investigation of CS_2 as a suitable solvent for 1,1,1-TCE should continue to determine if this solvent is capable of extracting 1,1,1-TCE from the blood at levels that are detectable with the GC-FID. Availabitity of a GC-ECD could eliminate this work since standard procedures for detecting 1,1,1-TCE using this instrumentation have already been determined.

c. The preliminary animal study should continue in order to verify that the CGRS is working effectively in vivo and to determine the ability of the semen analysis system to detect detremental effects to the animals' semen.

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FINAL REPORT

The Metabolism of 2-Methylheptane in Fischer 344 Rats

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Date:	13 September 89
Contract No:	F49620-88-C-0053

Metabolism of 2-Methylheptane in Fischer 344 Rats

by

J. Matthew Clemens

ABSTRACT

Fischer 344 Rats were intragastrically dosed with 2-methylheptane and the 48 hour urines collected. The urines were enzymes processed and then used for Gas Chromatographic (GC) metabolite analysis. Possible urine/metabolites were either purchased or synthesized in the lab. Urinary metabolite peaks were observed by comparing the GC tractings of dosed rats to those of control rats. The observed metabolite peaks were identified by matching their retention times to the retention times of metabolite standards with known structures. If the retention times were very similar, a small sample of urine was spiked with the standard to see if peak area grew. Four metabolites were identified in this manner. These metabolites were: 2-methyl-1-heptanol, 6-methyl-3-haptanol, 6-methyl-1,2-heptanediol, and 2-methyl-1,2-heptanediol.

ACKNOWLEDGEMENTS

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this researh. I also want to recognize Universal Energy Systems for their help in all of the administrative aspects of this program. Dr. Paul Serve' of WSU must be thanked for his advice and constant support throughout my research.

I would also like to thank the many people at AAMRL/THT that made my work there both rewarding and enjoyable. Special recognition goes to Dr. David Mattie, Mrs. K. O. Yu, and Dave Geiger for their invaluable assistance during this project.

I. INTRODUCTION

The Toxic Hazards Division of the Armstrong Aerospace Medical Research Laboratory at Wright-Patterson Air Force Base is responsible for evaluating the health hazards of chemicals that personnel handle or come in contact with. The pharmacological properties and pathways of metabolism are investigated, and from this information, tolerance levels of exposure are established.

The U.S. Air Force daily uses large quantities of hydrocarbons. The various uses of these hydrocarbons range from jet fuels to hydrocarbon based solvents. There exists the opportunity for Air Force personnel to be exposed to potentially large quantities of these vapors. It would be very beneficial to know the toxic effects resulting from exposure to these vapors.

During the early 1980's it was observed that unleaded gasoline caused renal damage in exposed rats. Further studies indicated that the nephrotoxic activity of unleaded gasoline occurred predominately from the fractions containing saturated, branched-chain aliphatic compounds (1). This finding initiated numerous studies of the component hydrocarbons in unleaded gasoline. Most of the exposure studies performed have been either long-term inhalation exposures or short-term oral dosings.

Several dimethyl and trimethyl octane isomers in gasoline have been found to be highly nephrotoxic. During short-term oral dosings, these compounds induced structural and functional changes in the kidneys of exposed male rats. Indications of renal damage were demonstrated by increased hyaline droplet formation and necrosis of the proximal tubular epithelium -p(2).

My research interests lie in the area of determining how structural differences such as chain branching affect the metabolism and toxicity of isomeric hydrocarbons. Previous

experience in characterizing urinary metabolites of cyclic hydrocarbons using gas chromatography contributed to my assignment to the Toxic Hazards Division of AAMRL.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Unbranched hydrocarbons such as n-octane do not induce nephrotoxicity. However, it has been demonstrated that branched chain hydrocarbons containing 8-12 carbons can ilicit a nephrotoxic effect in male rats. Currently, there are no reports indicating how the structure of alkane chain influences the nephrotoxicity of the hydrocarbon. It is hoped that by examining isomeric hydrocarbons, a relationship between branching structure and nephrotoxic effect can be developed.

The coal of this research is to document the degree of nephrotoxicity induced by an octane isomer containing a methyl group in the 2 or 3 position. 2-Methylheptane contains one branched methyl group in the middle of the molecule. The observed renal damage induced by 2-methylheptane will help determine if this mono-methyl branched octane isomer is as nephrotoxic as dimethyl or trimethyl octane isomers. An additional goal of this research is to determine which metabolites of 2-methylheptane are responsible for any kidney toxicity observed.

III.

a. Twelve male Fischer 344 rats were allocated to two exposure groups. The first group consisting of four rats was designated as a control group. This control group was intragastrically dosed with 1 ml/kg body weight of distilled water on alternating days. The remaining eight rats were orally dosed in a similar manner with 1 ml/kg body weight of neat 2-methylheptane. Following initial dosing, the rats were placed in metabolite cages for 38 hour urine collection, after which they were placed in plastic cages. The rats were weighed

daily. Water and feed were available ad libitum. For the remainder of the two week study, the rats were dosed on alternating days. On the final day of the study, the rats were fasted to facilitate pathologic observation. At the end of the 14-day dosing period, the rats were sacrificed by halothane overdose. The kidneys were harvested with one being preserved in 10% buffered formalin solution for histopathologic evaluation and the other homogenized to look for 2-methylheptane metabolites.

The collected 48 hour urines were isolated and treated according to the extraction technique of Yu (3). Equal volume aliquots from each urine sample were adjusted to a pH of 4.0. A 0.5 ml volume of glucuondase/sulfatse (calbiochem) was added to each aliquot and then incubated with shaking at 37°C for 24 hours. Following incubation, the urine samples were cooled to room temperature and then filtered separately through Clin-Elut tubes (Analytichen International) using methylene chloride as an eluent. The sample volumes were reduced to about 1 ml in preparation for gas chromatographic (GC) analysis.

A Hewlett-Packard 5880 gas chromatograph equipped with a flame ionization detector was used for metabolite analysis. A 30 m x .25 mm I.D. Carbowax 20 M fused silica capillary column was used. Urine samples from both the control and dosed groups were gas chromatographed.

Comparison of the GC tracings from both groups indicated new peaks at the following retention times: 5.55, 7.63, 14.36, 16.00, 17.68, 18.30, 20.21 minutes.

These potential metabolites were purchased from Wiley Organics:

2-methyl-1-heptanol

2-methyl-2-heptanol

2-methyl-3-heptanol

2-methyl-4-heptanol

6-methyl-3-heptanol

6-methyl-2-heptanol

The following potential metabolites were synthesized using referenced procedures.

2-Methylheptanoic acid was synthesized by the oxidation of 2-methyl-1-heptanol using CrO_3 and H_2SO_4 (4).

Five diols, 6-methyl-3,4-heptanediol, 6-methyl-2,3-heptanediol, 6-methyl-1,2-heptanediol, 2-methyl-1,2-heptanediol, and 2-methyl-3,4-heptanediol, were synthesized from the corresponding alkenes using OsO_4 as a catalyst (5).

6-Methylheptanoic acid was synthesized utilizing a two-step procedure. 5-Methyl-1 bromohexane underwent a substitution reaction with sodium cyanide to form the nitrile (6). The nitrile was then hydrolyzed to the corresponding acid using HCl (7). 6-Methyl-1-heptanol was obtained from the reduction of 6-Methylheptonoic acid by lithium aluminum hydride (8).

b. The purchased compounds and two synthesized diols, 6-methyl-1,2-heptanediol and 2-methyl-1,2-heptanediol, were analyzed by gas chromatography. The retention times were noted and compared to the metabolite peaks in the GC tracings of the dosed animal urine samples. Because the retention times did not correspond to any peaks in the GC

tracings, the following compounds are definitely not present as urinary metabolites of 2-methylheptane:

2-methyl-2-heptanol

2-methyl-3-heptanol

2-methyl-4-heptanol

6-methyl-2-heptanol

Several compounds were tentatively identified by spiking wire samples and observing peak growth in the GC tracings. These compounds and their retnetion times are:

2-methyl-1-heptanol	7.95	min.
6-methyl-3-heptanol	5.56	min.
6-methyl-1,2-heptanediol	20.27	min.
2-methyl-1,2-heptanediol	18.33	min.

Confirmatory testing by mass spectrometry is still pending.

IV. RECOMMENDATIONS:

Identification of metabolites in the kidney homogenates has not been performed. The method used for gas chromatographic analysis tends to cause excessive accumulation of non-mobile compounds at the column head. This unfortunate occurrence requires repeated cleaning of the chromatographic column which is a very slow process. Completion of this procedure could reveal which metabolites are responsible for the observed renal damage.

In a long term study, other octane derivatives could be examined to determine how chain branching structure affects metabolism and nephrotoxicity of homologous compounds.

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FINAL REPORT

Harness Belt Task

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USAF Researcher: Ints Kaleps, Ph. D.

Date: August 18, 1989

Contract number: F49620-88-C-0053

Same Report As
Prof. Joseph Szucs
(Report # 132)

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FINAL REPORT

A Study of Transport Delay Using an Aircraft Simulator: Pilot Study

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Date:	29 July 1989
Contract No:	F49620-88-C-0053

A Study of Transport Delay Using an Aircraft Simulator: Pilot Study

by

Lawrence Blair Fleischer

ABSTRACT

A study was designed to test the effects of Transport Delay on pilots who train using simulators. A high performance aircraft simulator was created. A side-step landing task, first used by Calspan Corp. to study of Transport Delay, was programmed into the simulation and used as the primary task. A scoring algorithm provided a performance measure as well as a means to give simulator users feedback to improve aircraft control. The purpose of this pilot study was to determine whether the information displayed from the scoring algorithm provided adequate feedback to allow participants to improve their score in subsequent trials.

There was a general improvement in scores as the test participants became accustomed to the simulator, however the improvement was not as large as expected due to the lack of qualitative feedback given with the performance scores. To improve feedback to participants, it was recommended graphic displays of the ground track and glide slope be provided in addition to the numeric overall score originally presented.

Acknowledgements

I wish to thank the Air Force Office of Scientific Research and the Air Force Systems Command for sponsoring this research. I would also like to thank AAMRL/HE for their support in recommending my appointment for the summer research position to Universal Energy Systems.

A special thanks must be extended to Captain Whiteley without whom I would not have had the opportunity for this research. This individual must also be commended in providing an enjoyable and rewarding atmosphere during my stay in Dayton as well as providing guidance throughout my research at Wright Patterson Air Force Base. To Matthew Middendorf, Bill Johnson, Steve Lusk and Cindy Soergel, who are the man power behind the Time Delay Study and Flight Simulator, thanks for your support.

To Laura, for encouraging me to come for the summer and keeping all of our tomorrows safe.

I. INTRODUCTION:

The use of high technology simulators has become an important tool in the training of pilots. These simulators are able to present aircraft controls and systems in a manner consistent with actual aircraft. To make the operating environment realistic, it is always desirable to use as many environmental details as possible. It is not surprising to find that the greater the amount of information presented in the visual scene, the better the display (Barnes,1978). Unfortunately, utilization of detailed displays causes significant computational problems for even the most sophisticated computer, resulting in a "sluggish" response of simulator controls. This "sluggish" response is known as time delay or **Transport Delay**. Using fewer details, such as rocks, trees, grass, and shades of color, reduce computational problems and do not necessarily compromise the utility of simulators. Use of geometrically modeled cues such as relative size, assumed size, height in the field, intersecting stripes, texture and linear perspective provide simulator users with appropriate and adequate perspective (Nelson and Richie, 1976). Stenger, Zimmerlin, Thomas and Braunstein (1981), note that shadows also play an important role in visual feedback for depth and motion.

A study was designed to test the effects on pilots who train using simulators that exhibit various degrees of Transport Delay. A high performance aircraft simulator made up of a Microvax 2 and 2 Silicon Graphics Iris 3120's was used. A side-step landing task, first used by Calspan Corp. (Knotts, 1986) to study of Transport Delay, was programmed into the simulation and used as the primary task. A scoring algorithm based on optimal ground track, aircraft speed and glide slope, provided a performance measure as well as a means to give simulator users feedback to improve aircraft control. The purpose of this pilot study was to determine whether the information displayed from the scoring algorithm provided adequate feedback to the simulator user, for improvement of

their score in subsequent trials.

My experience and training in the field of human factors and computer programming made me a logical choice for the summer program at AAMRL/HE. My studies of human-computer interface resulted in my assignment to the Transport Delay study.

II. OBJECTIVES OF THE RESEARCH EFFORT:

To determine if the information from the scoring algorithm and the information displayed provided adequate feedback to the simulator user, thus allowing improvement of their score in subsequent trials.

III. PROCEDURE:

Figure 1 displays the simulator environment. Participants were provided a control stick for the right hand and engine thrust controls for their left hand. The flight was displayed on a large screen television 6X10 ft in size, projected from behind and above the seat. In front of the seat was a color monitor simulating an instrument display which consisted of an Altimeter, Attitude Directional Indicator (ADI), Airspeed Indicator, Fuel Flow, Heading Indicator, and a Vertical Velocity Indicator (VVI) (Figure 2).

The simulation began with a fighter type aircraft on a landing approach 10,000 ft from the runway (Figure 3). Participants were to land the aircraft at the 1000 ft markers which were located just past the second set of Vertical Approach Slope Indicator (VASI) lights. Displayed on the screen in front of the participant were two runways. The aircraft was initially lined up on the right runway, indicated by a **green "X"** displayed in front of it (which disappears after a few seconds). As the participant continued on this approach, a **red "X"**, the side step cue, appeared in front of the aircraft within a 1000' window on approach to the runway, between 4000' and 5000'.

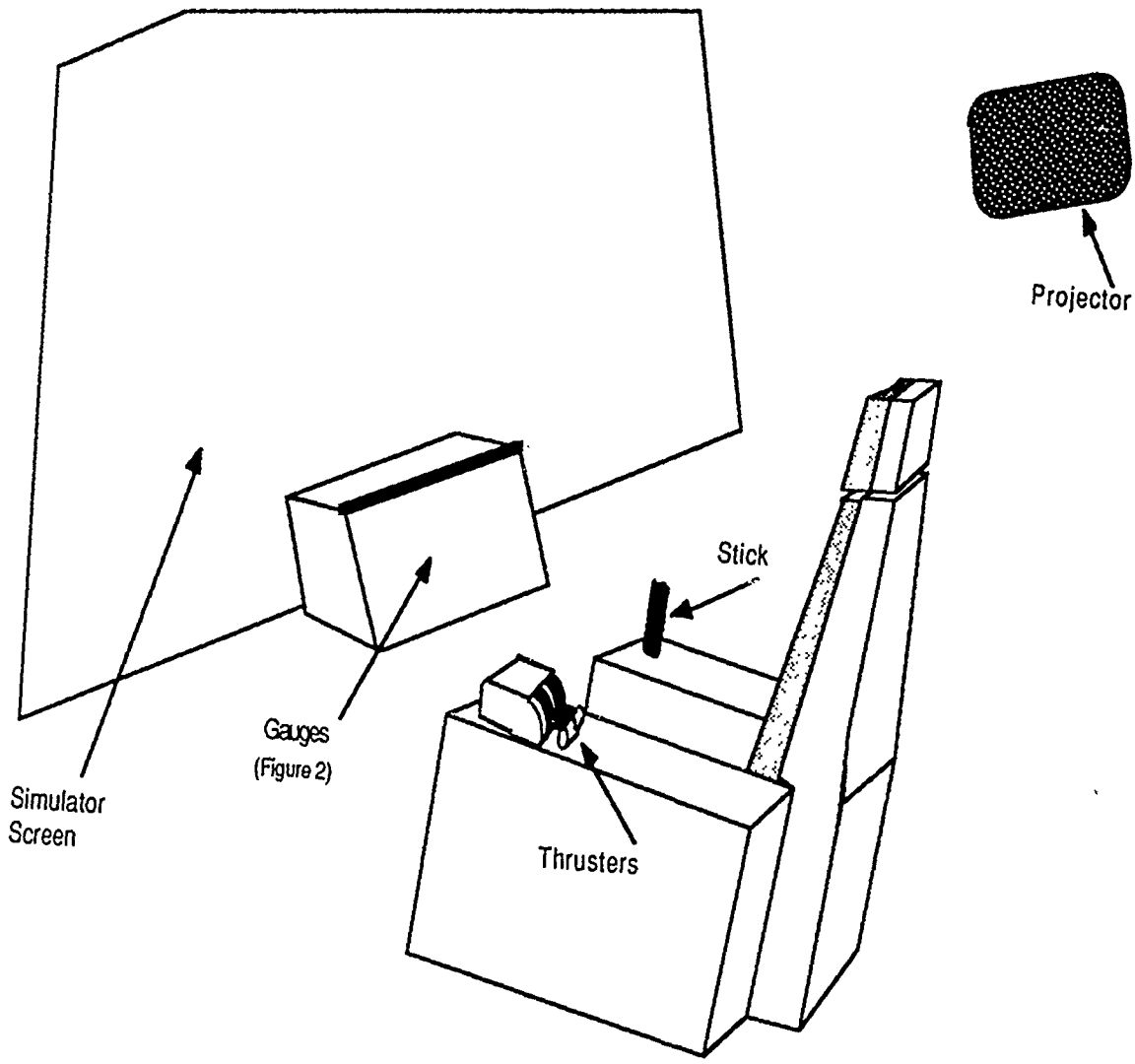

Figure 1. Simulator Environment

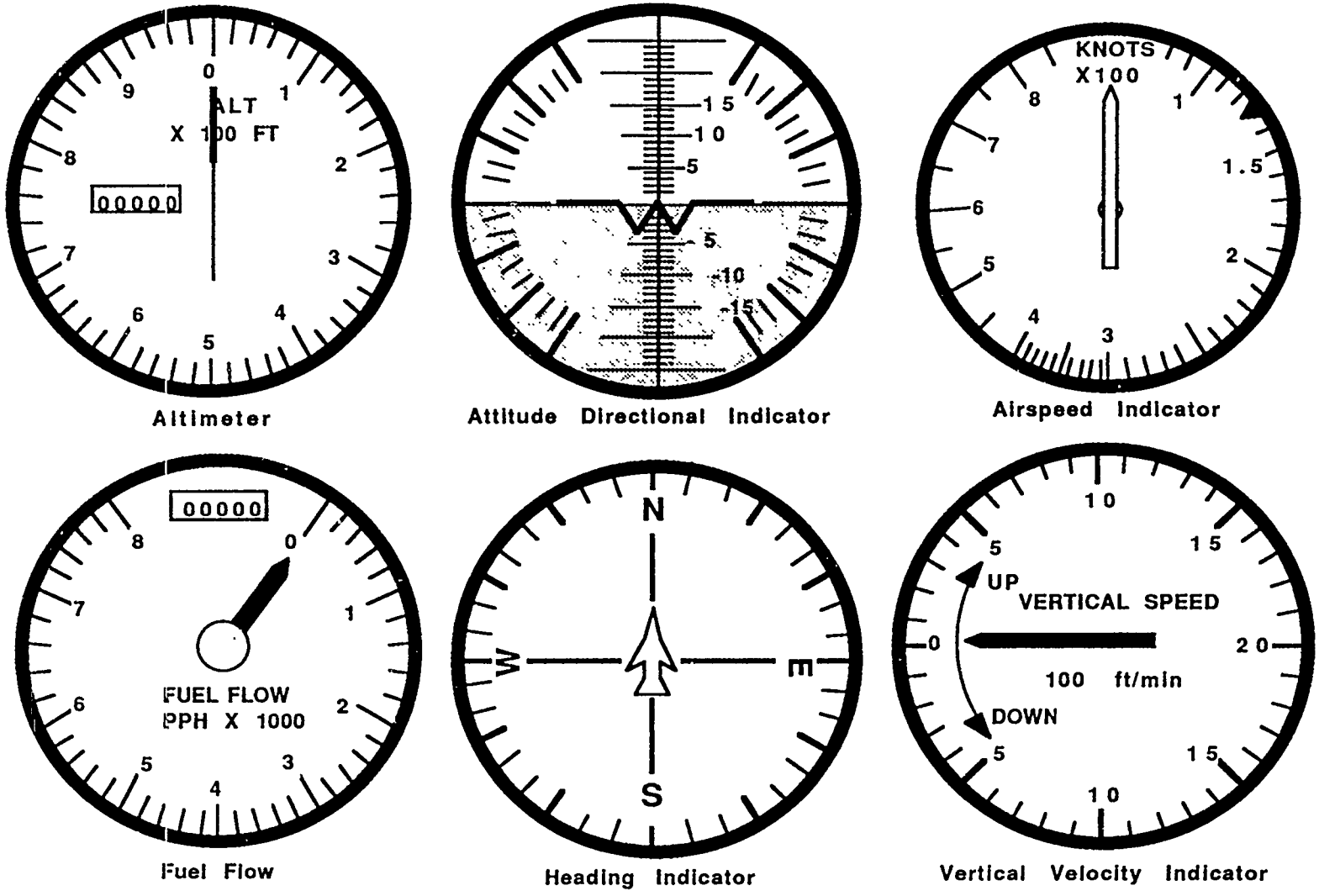

Figure 2. Instrument Display

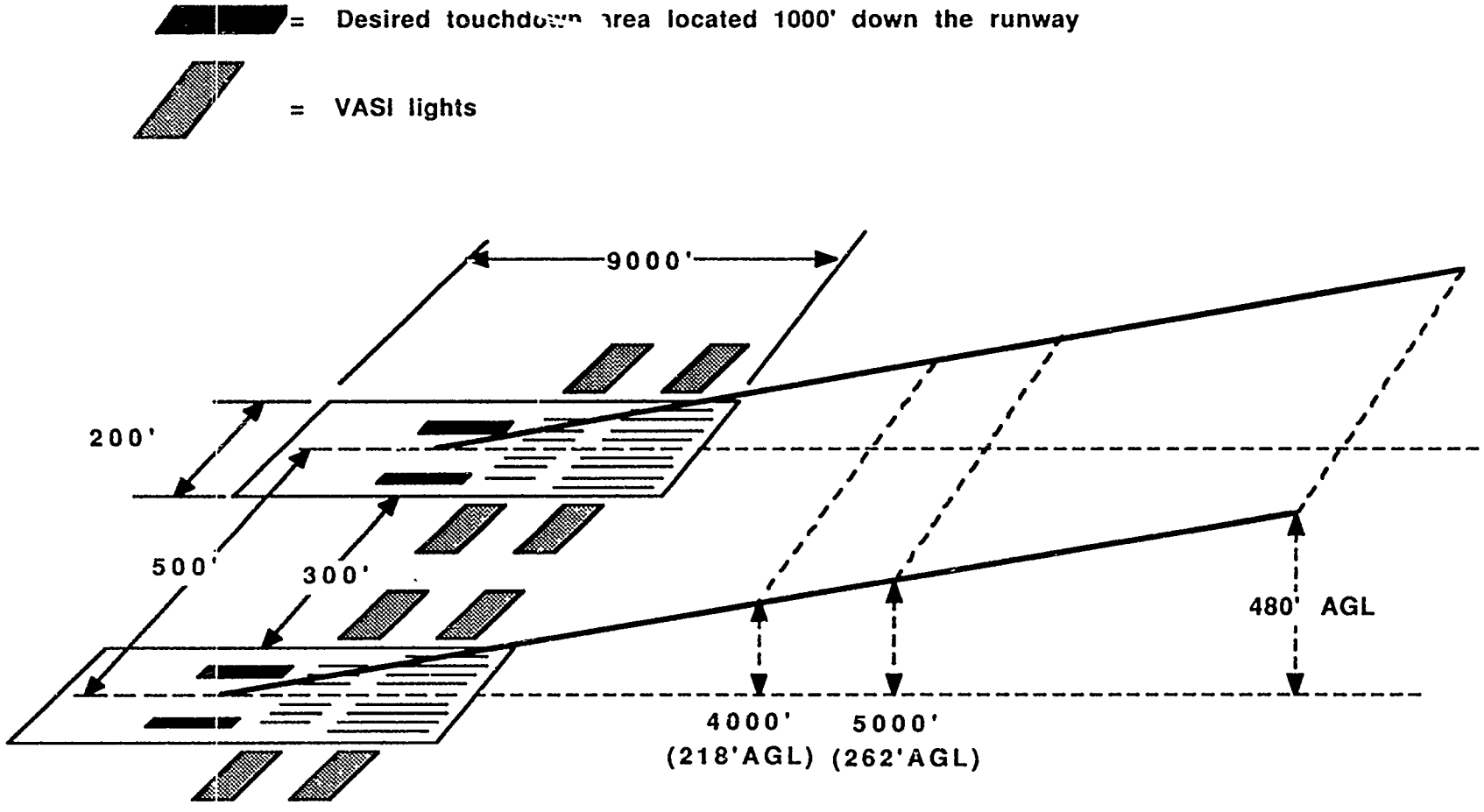

Figure 3. Runway Approach

The participant was then required to reacquire the left runway and land at a point just past the second set of VASI lights. Scores are presented on the large screen at the end of each trial to provide feed back for improvement on later trials (Figure 4). The information displayed were the means and standard deviations of the glide slope, ground track and air speed. A composite score was shown at the bottom of the screen for overall performance.

IV. EXPERIMENTAL DESIGN:

1. 40 Trials for pilot test.

2. 1 out of 5 approaches will be a "dummy" trial (i.e. a straight-in landing approach)

3. Two pilots and two non-pilots were used for the pilot study.

V. RECOMMENDATIONS:

There was a general improvement in scores as the test participants became accustomed to the simulator however, this improvement was not as large as expected due to the lack of qualitative feedback given with the performance scores. To improve feedback to participants, it was recommended graphic displays of the ground track and glide slope be provided in addition to the numeric overall score originally presented. Without this qualitative feedback there was no was to know where any problems occurred, just that the overall score was "high" and that problems did exist. Figure 5 shows the recommended changes to feedback provided to the participants after each trial. The solid line, in each case, represented the optimal flight while the dashed line represented the actual flight. Another pilot study will be run to test these new changes and other scoring algorithm changes made following this test.

Trial #1		
Glide slope	15.6	35.6
Line up	30.9	49.3
Air speed	10.2	6.1

Score = 147.7

Figure 4. Score Output

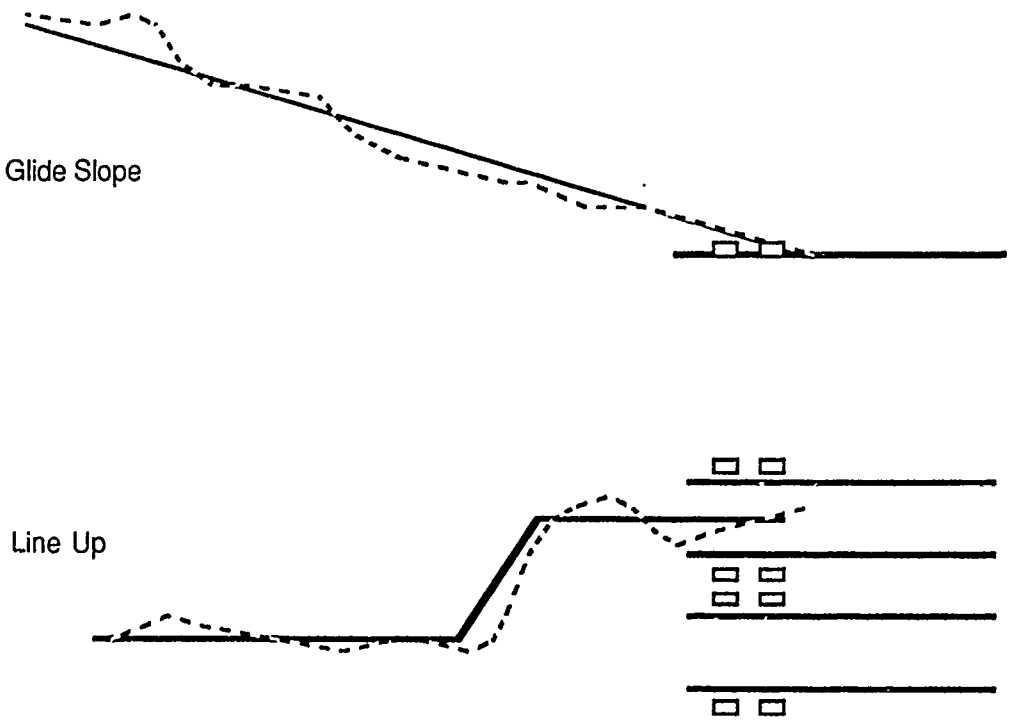

Figure 5. Recommended Graphics

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Appendix I.

Pilot Study Specifications

Flight Task Summary

- Begin flight at 10,000 ft (ground track) from ILS. A large **green "X"** will indicate initial alignment with corresponding runway; the cue will disappear within a few seconds.

- Begin scoring immediately.

.- Follow 2.5 degree glide slope, indicated by VASI, and maintain alignment with designated runway.

- When cued with large **red "X"**, switch to adjacent runway.

- Realign approach to adjacent runway. Continue approach until touchdown. Scoring will end at the runway threshold; data collection will continue through touchdown.

Initial Conditions

- Airspeed 133 knots (153 MPH) +/- 10 knots (Aerodynamic model trimmed at 25 degrees). The trial will never begin with airspeed of exactly 133 knots (optimal airspeed of aircraft for landing).

- Angle of attack 11 degrees.

- Landing gear down, flaps and speed brakes extended halfway.

- Altitude = 480 ft above ground level.

- Side step cue will occur within a 1,000 ft window between 4,000 and 5000 ft.

Scoring Output for User

1. Horizontal Error (ie. ground track/lineup) mean distance in feet and Standard Deviation.

2. Vertical Error (ie. glide slope) mean distance in feet and Standard Deviation.

3. Approach Speed Error (ie. airspeed) mean airspeed in miles per hour and Standard Deviation.

Output for Statistical Analysis

Landing

1. Scoring Output for User
2. Vertical velocity at weight on wheels.
3. Touchdown dispersion.
4. Attitude (roll and pitch angle).
5. Heading.
6. Position
7. Air speed

Step Response Characteristics

1. Damping.
2. Rise time.
3. Margin of stability.
4. Natural frequency

Pilot Induced Oscillation

1. Amplitude and duration

Time to Acquire Re-Allignment

1. Horizontal deviation of +/- 1 degree.
2. Vertical deviation of +/- 5 degrees.

Appendix II

Participant Briefing

Thanks for participating in this experiment. We're interested in operator performance, using a simulated high-performance aircraft, on a side-step landing task.

At the beginning of each trial, you will be on a final approach to land, in a landing configuration, including speed brakes, and on a 2.5 degree glide slope. Your angle of attack (AOA) will be 11 degrees, and will be displayed on the attitude directional indicator (ADI). Your initial altitude will be 480' above ground level (AGL), and will be displayed on the altimeter. Field altitude is 0 feet. Approach speed is 133 knots, and will be displayed on the airspeed indicator. The initial air-speed is perturbed such that you will need to make throttle adjustments in order to attain the desired approach for the task. When a throttle adjustment is made, there will be an associated change in engine noise and fuel flow (thrust). The fuel flow display will provide you with a measure of the amount of thrust. There is no wind disturbance and visibility is clear. You have 700 lbs. of fuel left and must land.

At the beginning of each trial, a large green "X" will indicate the runway with which you are currently aligned; the "X" will disappear after a few seconds. At some point you will be prompted by a large red "X" to begin your turn to the adjacent runway. At that time, switch to the adjacent runway and reattain the desired approach for landing. Continue your approach though the landing procedure. The objective is to land 1,000 feet past the beginning of the runway as indicated by the 1,000 foot bars. Some trials will be straight-in approaches and you will not have to side-step.

Ideal performance on this task will require you to maintain:

1) a glide slope of 2.5 degrees.

2) an airspeed of 133 knots indicated airspeed (KIAS), and

3) a ground track path close to the optimal path that will be measured against you.

The first diagram shows a 2.5 degree glide slope, with touchdown at 1,000 feet past threshold. To help you maintain this glide slope, you are provided with a visual approach slope indicator (VASI lights). [When on the proper glide slope, the top row of VASI lights will be red and the bottom row will be white. If you are too high, the top and bottom rows will be white. If you are too low, both rows will be red. A simple rhyme to help you remember this is: White over white, you're high as a kite. Red over red, your dead. Red over white you're flying all right.] Along with the VASI, you have a vertical velocity Indicator (VVI) to help you maintain an optimal rate of descent of 600 ft/min. The second diagram shows the optimal ground track that we will be measuring you flight performance against.

The scores that you will see after each trial are the means and standard deviations of the glide slope, line-up and air speed. There will be also a composite score generated from these same parameters.

You will have 40 trials broken into 4 sessions of 10, with short breaks (approximately 5 minutes) between each session.

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FINAL REPORT

MATERNAL TRANSFER OF HEXACHLOROBENZENE IN THE RAT

Prepared by:	Ellen S. Goldey
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Date:	28 Sep 89
Contract No:	F49620-88-C-0053

Maternal Transfer of Hexachlorobenzene in the Rat

by

Ellen S. Goldey

ABSTRACT

The uptake, distribution, and elimination of hexachlorobenzene (HCB) was assessed in nonpregnant, pregnant, and lactating rats, and their fetuses and suckling pups. Three weeks prior to breeding, virgin Sprague-Dawley rats were given a total oral dose of HCB in corn oil of 11 mg/kg body weight over three days. Concentrations of HCB were determined in the tissues from animals three weeks after dosing (at breeding), on day 21 of gestation and on postnatal (PN) days 7, 14 and 21. In all reproductive states of the dam, the fat had the highest concentration of HCB, followed by the liver, blood, kidney and brain. During pregnancy, the fetal blood and liver concentrations were 0.9 times the maternal blood HCB concentration, and the fetal brain HCB concentration was 0.5 times the dam blood HCB concentration. The maternal body burden of HCB was quickly depleted by lactational transfer of the HCB to the suckling pups as reflected in high HCB concentrations in the milk. By PN day 7, concentrations of HCB in dam tissues were approximately one third their initial concentrations, and by PN 14, HCB was only detected in the fat. On PN day 7, pup liver, blood, and kidney HCB levels were about 5 times the respective maternal tissue HCB levels, whereas the pup brain HCB concentration was 1.5 times the maternal brain HCB concentration. Tissue concentrations of HCB in nonpregnant females, measured at two week intervals over six weeks, showed a gradual decrease over the duration of the experiment.

ACKNOWLEDGMENTS

I would like to thank the Air Force Systems Command and the Air Force Office of Scientific Research for making this research program possible. Thanks also to Universal Energy Systems for coordinating the program so successfully.

Special thanks goes to the men and women of the Toxic Hazards division of the AAMRL. From the first day I was made to feel a part of the research team. The friendly and ready support of the technicians was invaluable in keeping the study running smoothly during the times when everything seemed to be happening at once. My sincere gratitude goes especially to Ssgt. Tim Whittaker for his calm, steady support, his technical expertise and his good humor.

Finally, I would like to recognize the friendly interest and enthusiasm that Dr. Jeffrey Fisher showed for this project. He always welcomed questions and his advice proved invaluable in the avoidance of numerous stumbling blocks. I thoroughly enjoyed working with him.

I. Introduction

Hexachlorobenzene (HCB) is a widespread environmental contaminant. The U.S. EPA reported that the major route of exposure to HCB is probably in the diet (U.S. EPA, 1987). When ingested, HCB is readily absorbed across the intestine and distributed to all tissues (Matthews, 1986). It concentrates in adipose tissue and is metabolized very slowly.

Numerous studies have shown that HCB is a common residue found in human tissues. Ansari et al. (1986) analyzed fat tissue collected from 109 bodies at autopsy and found HCB in all samples at levels ranging from 18-35 ng/g of fat. Mack and Mohadjer (1985) detected HCB in nearly 100% of the adipose tissue samples collected from the U.S. population from 1970-1983.

Chlorinated compounds, including HCB, have been reported at high levels in milk from lactating women (Stacey et al., 1985). Hexachlorobenzene is a highly lipophilic compound and it has been shown to accumulate in the adipose tissue. During pregnancy, fat accumulates in the body and is then utilized for milk production in the milk gland during late pregnancy and lactation. Of particular interest in this study was the ability of HCB to mobilize with the fat during these reproductive states.

The current project is relevant to the Installation Restoration Program. This research program is aimed at solving risk assessment problems associated with clean-up of landfills on Air Force bases. Hexachlorobenzene is a common long-lived contaminant in the environment as well as in the body. Consequently, exposure is widespread and the bioaccumulation of this compound may represent a health risk. The Toxic Hazards Division (THA) of the Armstrong Aerospace Medical Research Laboratory is interested in toxicokinetics as it relates to pregnant women and those with breast-feeding infants. Currently emphasized in this division is the development of pharmacokinetic models to be used in risk assessments for women who are potentially exposed to xenobiotics in the workplace and through bioaccumulation from the environment.

My master's research project stressed the use of behavioral testing procedures to determine the teratological effects of trichloroethylene (TCE) on the central nervous system in rats. Included in my previous research experience was the collection of tissues from rats exposed to TCE and the subsequent detection of TCE levels in these tissues using gas chromatography.

These data were used to support the development of a pharmacokinetic model for pregnancy and lactational transfer of TCE. This experience contributed to my assignment to the Toxic Hazard Assessment branch of the AAMRL for the current project.

II. OBJECTIVES OF THE RESEARCH EFFORT

a. The primary objective of this study was to quantitatively evaluate the bulk transfer of HCB from the pregnant and lactating rat to the offspring. My assignment as a summer researcher was to determine the tissue concentrations of HCB in the maternal liver, kidney, brain, blood, fat and milk, as well as the concentrations in the pup liver, kidney, brain and blood.

b. To assess the major target tissues for storage of HCB and to study the time course of the release of the chemical from the dam to the developing offspring.

c. To compile a database that will be useful for the development of a pharmacokinetic model for the the fate of HCB during pregnancy and lactation. A tissue dosimetry study provides partition coefficient information and a clear indication of major target compartments to be used in the development of the model.

III. METHODS

a. Hexachlorobenzene was dissolved in corn oil, and total dosage of 11 mg/kg was administered to 60 rats over 3 days by gavage (volume based on 2ml/kg) two weeks prior to breeding. This allowed the body burden of HCB to compartmentalize and stabilize in the rats' tissues. Rats were weighed twice weekly, and food and water were available *ad lib.* Rats were bred, and the day when sperm were found in the vaginal tract was designated as day zero of gestation. Litters were culled to eight pups at birth.

b. Groups of 6-10 rats and their offspring were sacrificed at designated stages of gestation and lactation (Table 1) to determine maternal concentrations of HCB in the blood, perirenal fat, brain, liver, kidney, and milk, fetal concentrations of HCB in the liver, brain, and blood, and pup HCB concentrations in the liver, kidney, brain, and blood. Hexachlorobenzene was extracted from the tissues by homogenizing the tissue in 10 vol. of hexane, centrifuging the homogenate and assaying each sample in triplicate by direct injection onto a Hewlett Packard (5890 series) gas chromatograph equipped with a Deactiglas column packed with 3% OV-101 W-HP 100/120 (3 feet long, 2mm ID) and a ^{63}Ni electron capture detector. Percent recovery was determined for each tissue type.

On day 21 of gestation, six dams were sacrificed for tissue analyses, and HCB levels were determined in the above tissues and in fetal liver, brain and blood.

c. Additionally, five female rats were given a single 3.7 mg/kg dose and the oral uptake of HCB was determined from blood samples collected at 10 min., 30 min., and 1 hr., 2 hr. and 3 hr. intervals. Blood was collected from the tail vein of each rat after immobilizing the animal in a plastic restraint cylinder. A heparinized microhematocrit capillary tube was fitted into the base of a 25-gauge disposable needle, and the needle was inserted into the lateral vein of the tail. Blood filled the tube by capillary action (70 ul). Samples were taken from distal to proximal locations along the length of the tail.

IV. RESULTS

a. The results of this study are represented in Tables 1, 2, 3 and in Fig. 1.

b. The HCB concentrations declined gradually in the tissues of the nonpregnant rats (Table 1). There was littie effect of pregnancy on the concentrations of HCB in the dam (Table 2) compared to concentrations in nonpregnant rats. There was, however, a sharp decrease in maternal tissue concentrations during lactation. By day 14 PP, concentrations were near the level of detection for all tissues but fat. Milk concentrations at day 21 PP were not detectable and the rest of the tissues from this stage remain to be analysed.

c. The bulk transfer phenomenon is clearly depicted in the rapid increase of HCB tissue concentraion in the suckling pups (Table 3). While the HCB concentration in fetal tissue reflects the maternal blood concentration, there is a surge in pup tissue concentrations by day 7 PP. It is apparent that this increase is due to the transfer of HCB from fat stores in the dam to the milk.

d. The oral uptake of HCB reaches a maximum at approximately 2 hours post gavage (Fig. 1).

V. RECOMMENDATIONS

a. The results of this study will be used in the development of a physiologically based mathematical model for the maternal transfer of HCB. This work will be useful to establishing risk assessment guidelines for HCB and other xenobiotics of similar structure and activity.

b. The results will also be used as part of a larger study to determine the teratology of HCB. Since developing organisms are often more susceptible to the effects of toxins, I believe that it is important to assess the effects of a compound on maternally exposed offspring. Specifically, I will focus on the effects of HCB on the brain, the eye, and numerous types of behavior in order to assess the neuroteratology of this compound.

Table 1: Mean HCB tissue concentration (ng/g) ± S.E. from nonpregnant rats 3, 5, and 7 weeks following dosing. N indicates the number of dams analysed.

week (N)	Liver	Kidney	Brain	Blood	Fat
3 (8)	1209.50 ± 70.46	622.66 ± 77.56	616.06 ± 33.58	712.96 ± 31.49	28385 ± 1848
5 (8)	777.78 ± 69.07	391.36 ± 52.71	466.86 ± 32.04	362.04 ± 24.23	18920 ± 1213
7 (7)	625.45 ± 55.64	323.58 ± 26.57	377.38 ± 18.19	396.13 ± 32.24	17727 ± 1038

Table 2: Mean HCB tissue concentration (ng/g) ± S.E. from dams during gestation and 7 and 14 days post partum (PP). N indicates the number of dams analysed.

stage (N)	Liver	Kidney	Brain	Blood	Fat	Milk
gest. (5)	566.78 ± 58.72	428.83 ± 46.39	304.18 ± 30.11	267.20 ± 7.46	17276 ± 2180	
PP 7 (10)	187.65 ± 12.35	108.39 ± 7.11	117.96 ± 7.87	126.16 ± 14.62	11582 ± 6035	547.62 ± 39.45
PP 14 (11)	33.25 ± 3.18	28.45 ± 2.81	26.56 ± 2.61	NA	423 ± 90	17.43 ± 5.92

Table 3: Mean HCB tissue concentration (ng/g) ± S.E. from fetuses during gestation and from pups 7, 14, and 21 days post partum (PP). N indicates number of litters (4 pups/litter analysed).

stage (N)	Liver	Kidney	Brain	Blood
fetus (5)	217.63 ± 16.81	NA	117.05 ± 9.79	212.04 ± 10.42
PP 7 (10)	1045.51 ± 42.35	492.94 ± 24.34	174.23 ± 10.18	655.41 ± 48.77
PP 14 (11)	330.03 ± 17.78	200.15 ± 12.53	133.22 ± 6.68	243.16 ± 15.46
PP 21 (10)	200.79 ± 18.79	91.03 ± 8.88	101.14 ± 16.22	136.03 ± 10.09

NA denotes data not analysed.

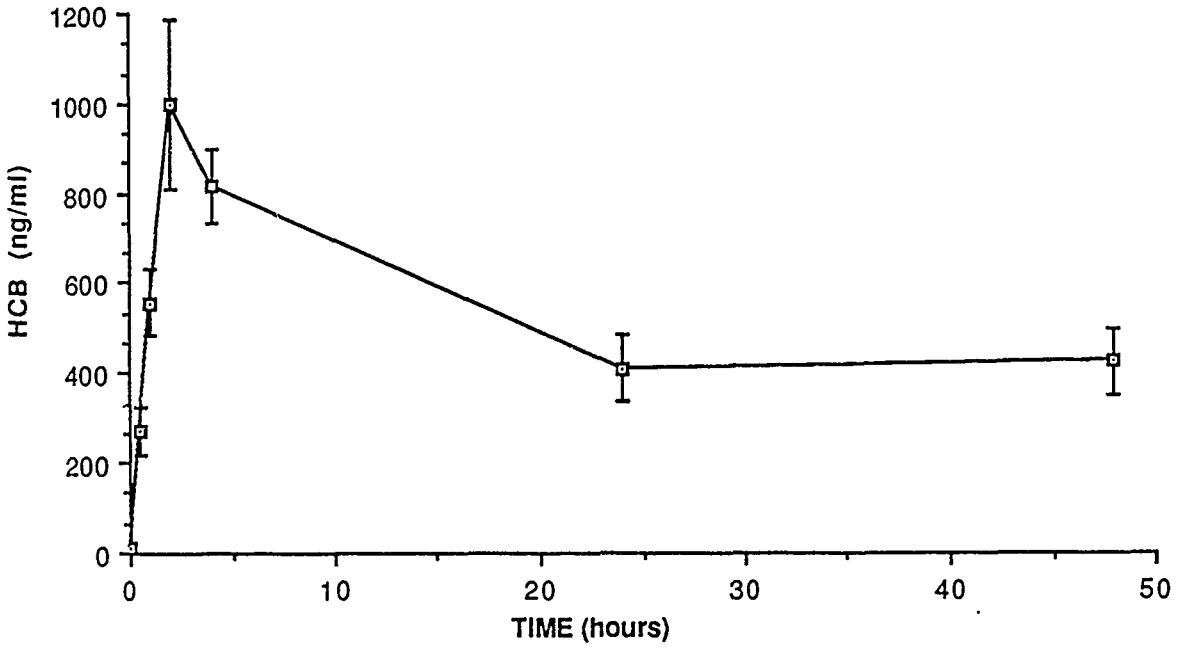

Fig. 1: Oral uptake of HCB (± S.E.) in female rats given 11 mg/kg HCB in corn oil via gastric intubation (N=5 rats).

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FINAL REPORT

EFFECTS OF DATA ERROR ON PROBLEM-SOLVING HEURISTICS

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Contract No:	F49620-85-C-0013

Same Report As
Prof. Bonnie Walker
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FINAL REPORT

THE PHYSIOLOGICAL EFFECTS OF DOBUTAMINE ON THE CARDIOVASCULAR SYSTEM

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Date: September 8, 1989

Contract No: F49620-88-C-0053

THE PHYSIOLOGICAL EFFECTS OF DOBUTAMINE ON THE CARDIOVASCULAR SYSTEM

by

Deborah E. Hollenbach

ABSTRACT

Dobutamine is a pharmacological agent that acts on beta-1 receptors in an inotrophic manner to increase myocardial contractility. Due to this action, dobutamine has been selected as a possible agent to be used to decrease the body's susecptability to G/LOC. G/LOC occurs when there is a critical reduction of cerebral blood flow as a result of increased G-force. Dobutamine (0, 20, 40, 60 mg/Kg/day) was prepared in normal saline and infused for two hours a day, five days a week, for six weeks, into miniature swine. The animals showed a significant dose-dependent increase in heart rate during infusion. The heart rates were 62 $\pm$2, 144 $\pm$ 2, 172 $\pm$ 2, and 181 $\pm$ 3 during the infusion process. Systolic blood pressure (110 $\pm$ 3, 99 $\pm$ 2, 86 $\pm$ 2 and 77 $\pm$ 3) and diastolic blood pressure (68 $\pm$ 2, 56 $\pm$ 2, 43 $\pm$ 2, and 40 $\pm$ 2) decreased in a similar dose-dependent manner.

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I specifically wish to thank Dr. James Cooper for allowing me the opportunity to work with him and his fellow researchers. Most importantly, I wish to express my appreciation for the time and effort that he spent in teaching me techniques that will prove invaluable to me as I continue on through vet school. The advice and the opportunities to improve my skills are invaluable to me.

I also wish to thank Dr. Beverly Girten for allowing me to assist her and Dr. Cooper with the G-tolerance project and to use freely of the background information provided in the protocol for writing this paper. I wish to express my gratitude to Susan Young for her advice on many topics and her assistance during the infusion of the pigs. Finally, I wish to thank Dr. Mayer, Dr. Ballinger, Tim Bausman and Brenda Schimmel for the many things that they taught me over the summer.

I. INTRODUCTION:

Introduction of the F-15 and F-16 aircraft into the Air Force inventory has centered on the reality that factors limiting the speed and maneuverability of fighter aircraft are not entirely dependent on the design of the airframes but rather rely heavily upon the physical limitations of the pilot. Specifically important is the pilots susceptibility to G-induced loss of consciousness (G/LOC). The Air Force, therefore, has a requirement for basic research data regarding methodologies which may be employed to enhance a pilots G-tolerance.

As a second year veterinary student, I desire to work with a variety of animal species and to explore opportunities in lab animal veterinary medicine. Having completed an extensive study of anatomy and the basics of pharmacology, I felt qualified to assist with the first phase of the G-tolerance project. In addition, previous work and a M.S. thesis research project in the area of drug delivery systems and behavior toxicology has taught me to conduct basic research.

II. OBJECTIVES:

The objective of the entire research project is to determine if the pharmacologic agent dobutamine will increase G-tolerance in the pig. My specific objective is to generate basic research data by determining the

physiologic effects of infused dobutamine on the cardiovascular system of the pig. I will do this by looking at its effects on respiration rate, heart rate and blood pressure.

III. TECHNICAL BACKGROUND:

G/LOC has been defined as "a state of altered perception wherein (one's) awareness of reality is absent as a result of a sudden, critical reduction of cerebral blood flow caused by increased G-force" (Burton, 1988). This inability of the cardiovascular system to supply blood to the brain under G can be divided into two parts. The first involves the inability of the heart to generate the substantial intraventricular pressures needed to force blood into the brain against hydrostatic fluid pressures experienced by the pilot (Lamb, 1960). The second involves inadequate return of blood to the heart caused principally by pooling of the blood in the extremities. To date, the efforts proposed to relieve G/LOC have been aimed at preventing the pooling of blood and facilitating venous return to the heart. These methods have involved the use of anti-g suits and implementation of the anti-G straining maneuver (AGSM).

The realization that AGSM is probably the most successful means of combating G/LOC currently available has led to studies aimed at determining the type of exercise

program which would most effectively reduce the extreme fatigue which accompanies AGSM. Research by Epperson in this area demonstrated that 11-12 weeks of resistance training intended to increase muscle mass appears to show the most promise in reducing AGSM-associated fatigue (Epperson, 1982). This type of exercise has been shown to result in both muscle hypertrophy and an improved muscle capacity to conduct anaerobic metabolism (Burton, 1987). Both of these attributes enhance the effectiveness (force) of the anti-G straining maneuver and lengthen the period of time for which it can be maintained. Vigorous aerobic exercise, on the other hand, appears to be contrarelated to the development of G-tolerance (Johanson, 1988). This may be due to the propensity for aerobic exercise to increase the size of capillary beds present in muscle tissue. It is theorized that these capillaries provide additional vascular areas available for venous pooling during G, thus counteracting any positive effects associated with enhanced cardiac performance. Resistance training does not increase the number of capillaries per unit of muscle tissue (Tesch, 1984).

Dobutamine is a relatively new synthetic catecholamine employed clinically in the treatment of congestive heart failure. Dobutamine is particularly well suited to the present application in that it possesses marked beta-1

agonist activity characterized by significantly increasing cardiac contractility while, at therapeutic doses lacking the systemic vasodilatory activity associated with beta-2 receptor stimulation (Leier, 1982) The improvements in cardiac function seen in patients with heart disease who have been treated with dobutamine appear to persist for several weeks following withdrawal of the drug indicating these effects may be due to persistent physical or chemical changes in the muscle cells (Liang, 1984).

In 1979, Liang demonstrated that the daily intravenous infusion of dobutamine into dogs over a five week period resulted in a decrease in resting heart rate, enhanced myocardial contractility and decreased levels of serum lactate and endogenous catecholamines. He also observed indications of enhanced ability of the heart to utilize oxygen. These findings are similar to those seen in a human study where dobutamine appeared to increase the aerobic enzyme activity in skeletal muscle cells of bed rest patients (Sullivan, 1986). Liang suggests that chronically elevated serum catecholamine levels result in increased cardiac performance.

In summary, chronic infusion of dobutamine has been shown to mimic the effects of exercise in that it increases cardiac performance and increases the capacity of skeletal muscle systems to extract oxygen from the blood,

Theoretically this combination of effects could be beneficial in preventing G/LOC by facilitating the heart's ability to maintain an adequate blood supply to the brain and, at the same time, augmenting the pilots ability to sustain the anti-g straining maneuver.

IV. EXPERIMENTAL DESIGN:

This study requires the intact cardiovascular and muscular systems along with the biochemical machinery necessary to metabolize the pharmacological agent in question. Therefore the study will involve the use of minerature swine as animal models for humans. The pig was chosen because its cardiovascular system is anatomically and physiologically similar to man, and the pig has been shown to elicit the AGSM spontaneously when subjected to G stress (Hughes, 1986).

A. Catheterization Technique:

The swine must have catheters placed and maintained for the intravenous administration of dobutamine for a seven week period. The swine were preanesthetized with ketamine (20 mg/kg) followed by surgical anesthesia with a mixture of isoflurane, nitrous oxide and oxygen.

Placement of the catheter was a slight variation of the procedures described by Witt (Witt, 1980). A skin incision was made in the ventral midline of the neck and the external jugular vein was isolated. A silastic catheter was inserted

five inches into the vein and sutured in place with silk sutures both proximal and distal to the point of insertion. A two centimeter by one centimeter patch of dacron was glued to the catheter and sutured to the surrounding muscle to secure the catheter into place. The free end of the catheter was then passed subcutaneously to a point on the midline of the dorsal surface of the neck and exteriorized. The pigs were placed into cloth and belt jackets which protected the catheters. A five inch by four inch removable square over the dorsal surface of the pig allowed for easy access to the catheter.

Once in place, the catheters were flushed every other day until the start of the experiment with ten milliliters o [illegible]rinized saline and a two milliliter swine flush (50 ml de [illegible]e, 1.24 ml heparin, 2 ml gentamicin). Bicillin (1 ml) [illegible]tered immediately following the catheter placement surgery.

B. Primary Study:

One week following surgery, sixteen pigs (four per group) were restrained in Panepinto slings and were infused for a period of seven weeks with one of the following doses of dobutamine (0,20,40,60 mg/Kg/day) in normal saline. They were infused slowly over a two hour period (50 ml/hr). During the first week the dosage was one half of the intended dose to slowly acclimate the heart to the chemical

in order to avoid stressing the heart muscle unnecessarily. Prior to daily infusion a resting respiration rate, heart rate and blood pressure was determined. The same parameters were recorded at the end of both one and two hours of infusion.

Following a total of thirty days of infusion at the desired dosage, the pigs were withheld from infusion for twenty-four hours and then prepared for the G-tolerance portion of the project. This portion of the project was conducted by other investigators and will not be included in this report.

C. Statistics:

Each dosage group contained four animals. The respiration rates, heart rates and blood pressures were analyzed using one-way analysis of variance. In the event a significant F value was found, Tukeys post hoc test was used to make pairwise comparisons. $P<0.05$ was accepted as the level of significance.

V. RESULTS AND DISCUSSION:

A. Effect on Respiration Rate:

Dobutamine infusion produced no effect on the respiration rate of the sixteen pigs. This was as expected since dobutamine evokes its action at the level of beta-1 receptors, which are located primarily in heart muscle.

B. Effect on Heart Rate:

Figure 1 shows the effect of 0, 20, 40, 60 mg/Kg/day dobutamine on the average heart rate of the infused pigs. Average heart rates of 62 ± 2, 144 ± 2, 172 ± 2, and 181 ±3 beats per minute were recorded after one and two hours of infusion. There were no significant differences between the two readings, but in all cases (except the controls) the heart rates were significantly higher than the baseline heart rates. The values recorded for each dosage group were significantly higher than the values recorded for the preceding group, in a dose-dependent manner.

Figure 2 shows the comparison of the baseline heart rates at weeks one and six. For each dosage group, the values at week six were significantly lower than those at week one. This may reflect the acclimation of the pigs to the slings and the infusion routine. There was a significant difference ($p=0.01$) between the dosage groups at week six. Further analyzation revealed that the control animals (0 mg/kg/day dobutamine) showed a significantly higher baseline heart rate over the other dosage groups. This is in accordance with the bradycardia observed at rest by both Davidson (1988) in rats and Liang(1979) in dogs chronically infused with dobutamine for six weeks. It is also seen as a result of physical training and conditioning (Liang, 1979).

C. Effect on Systolic Blood Pressure:

Figure 3 shows the effect of infused dobutamine on the systolic blood pressure of pigs. Systolic blood pressure readings at hour one of 110 ±3, 99 ± 2, 86 ± 2 and 77 ± 3 were recorded for the 0, 20, 40, 60 mg/kg/day dobutamine dosage groups. There were no significant differences between the pressures at hour one and hour two. All one and two hour readings (except for control animals) were significantly lower than the baseline values. In addition, each dosage group showed an average systolic pressure significantly lower that of the preceding dosage group, in a dose-dependent manner.

D. Effect on Diastolic Blood Pressure:

Figure 4 shows the effect of infused dobutamine on the diastolic blood pressure of the pigs. For each dosage group (0, 20, 40, 60 mg/kg/day dobutamine) there are no significant differences between the pressures at hour one and two. These values (68 ± 2, 56 ± 2, 43 ± 2, and 40 ± 2) are significantly lower than the baseline values in all cases (except for control animals). There were significant differences between the infusion readings of the 0, 20 and 40 mg/kg/day dobutamine dosage groups, but there was no significant difference between the values of the 40 and 60 mg/kg/day dobutamine dosage groups. This appears to indicate that dobutamines decrease diastolic blood pressure

in a dose-dependent manner, but only to a certain level. After that level has been reached, diastolic blood pressure no longer decreases.

E. Conclusions:

Dobutamine's activity occurs via the inotrophic stimulation of beta-1 receptors of the heart. These receptors serve the muscle fibers involved in myocardial contractility. It does not have activity at the beta-2 receptors, which are involved with peripheral vasodialation. For this reason, dobutamine is more specific in its action than the pharmacologic agent, isoproterenol, which acts at both beta-1 and beta-2 receptor sites. By acting selectively at the beta-1 receptors, dobutamine can increase myocardial contractility, as evidenced by the dose-dependent increase in heart rate, without increasing peripheral vasodilation. An increase in peripheral vasodilation could lead to the vascular pooling that often ends in G/LOC.

Dobutamine increased the heart rate of the pigs in a dose-dependent manner. The heart rates remained elevated during the daily two hour infusion period. While increasing the heart rate, the systolic and diastolic pressures decreased significantly in a dose-dependent manner. The only exception to this was the diastolic pressure in the high dosage (60 mg/Kg/day dobutamine) group. It was not significantly higher then that of the animals receiving 40 mg/Kg/day.

Based on the data obtained during this portion of the G-Tolerance project, dobutamine appears to increase heart rate in a manner similar to physical training and conditioning. The increase in heart rate and the decrease in systolic and systolic blood pressures observed after six weeks of dobutamine infusion appear to be a positive and encouraging finding. Additionally, bradycardia at baseline levels was observed in the dobutamine-infused groups, as has been seen in experiments involving both physical training and dobutamine infusion in rats and dogs (Davidson, 1986, Liang, 1979). While it was hoped that a decrease in baseline heart rate would have resulted from the drug, the decease which was actualy observed may have been primarily due to a condition effect, based on the fact that it was also observed in the controls.

IV. RECOMMENDATIONS:

Continued experimentation with the pharmacologic agent, dobutamine is necessary before all of the effects will be Known. Based on the data obtained in this portion of the project, the increased heart rates and decreased systolic and diastolic blood pressures should condition the heart in the same manner as 11-12 weeks of physical conditioning. The dose-dependent manner of the increases in heart rate shows promise of creating a range of dobutamine influence. The next logical step is to see if the dobutamine infusion does indeed have an effect on G-tolerance in the pig.

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FIGURE 1:AVERAGE HEART RATE

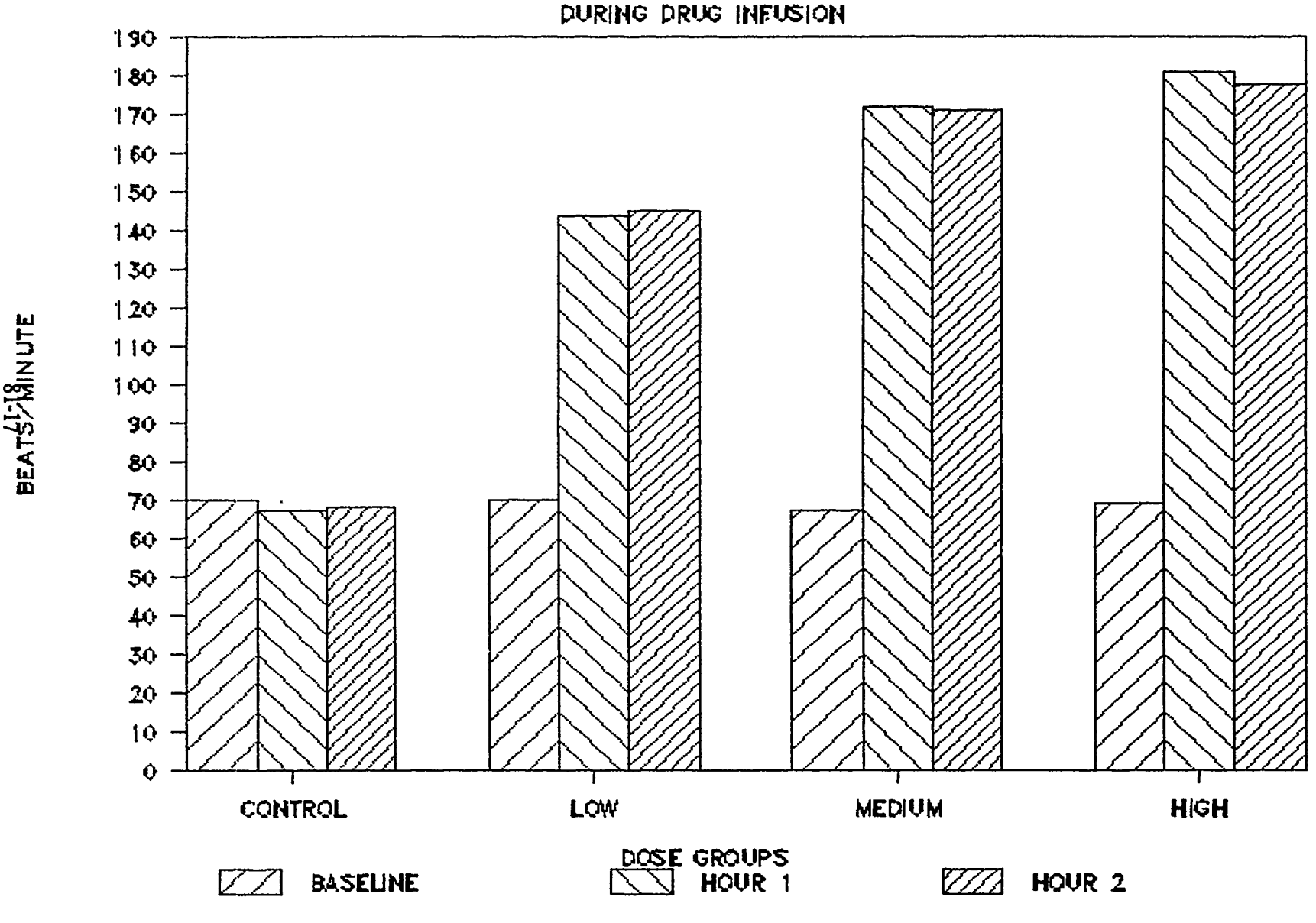


FIGURE 2: BASELINE HEART RATE

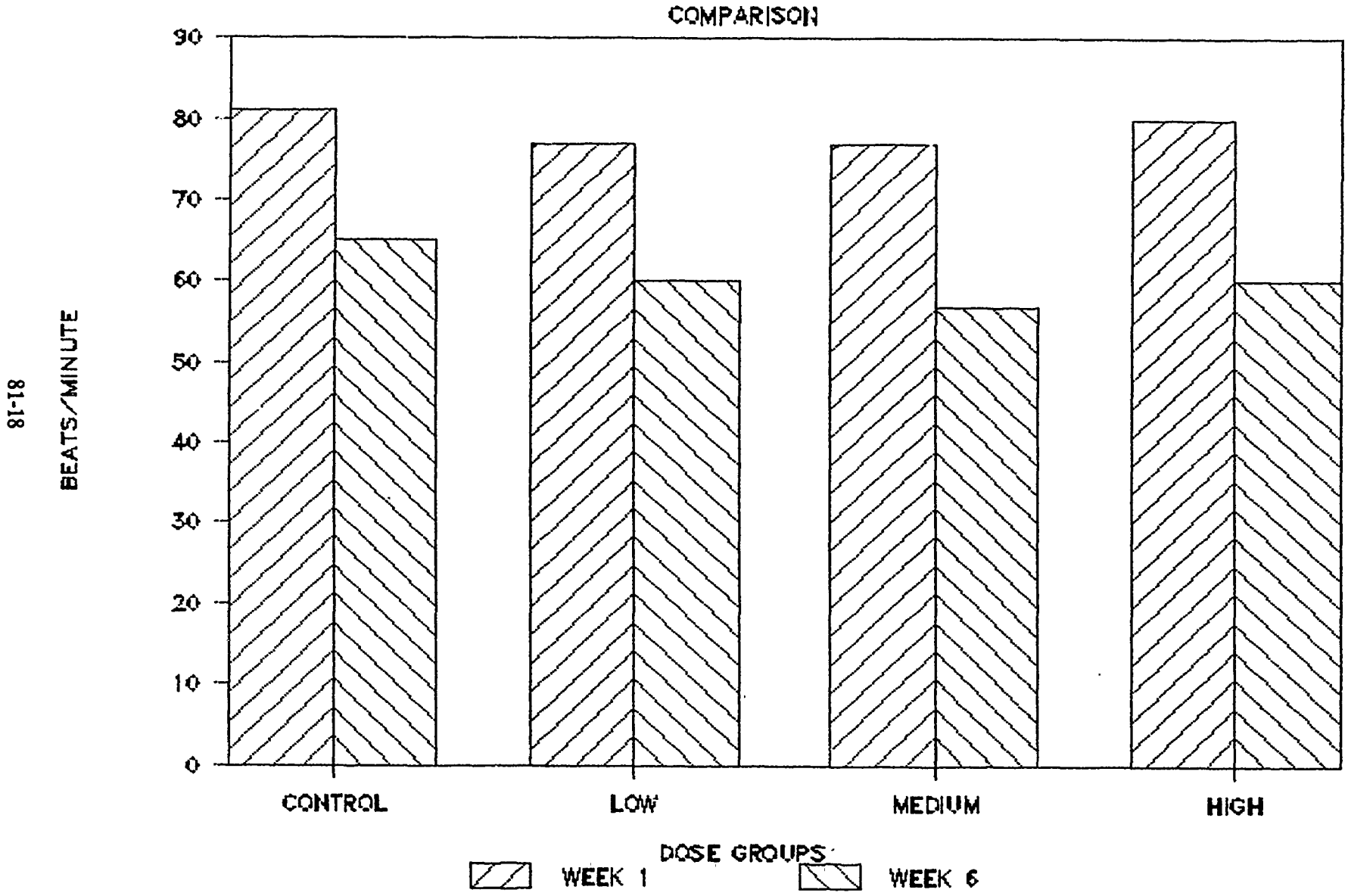


FIGURE 3: AVERAGE SYSTOLIC BP

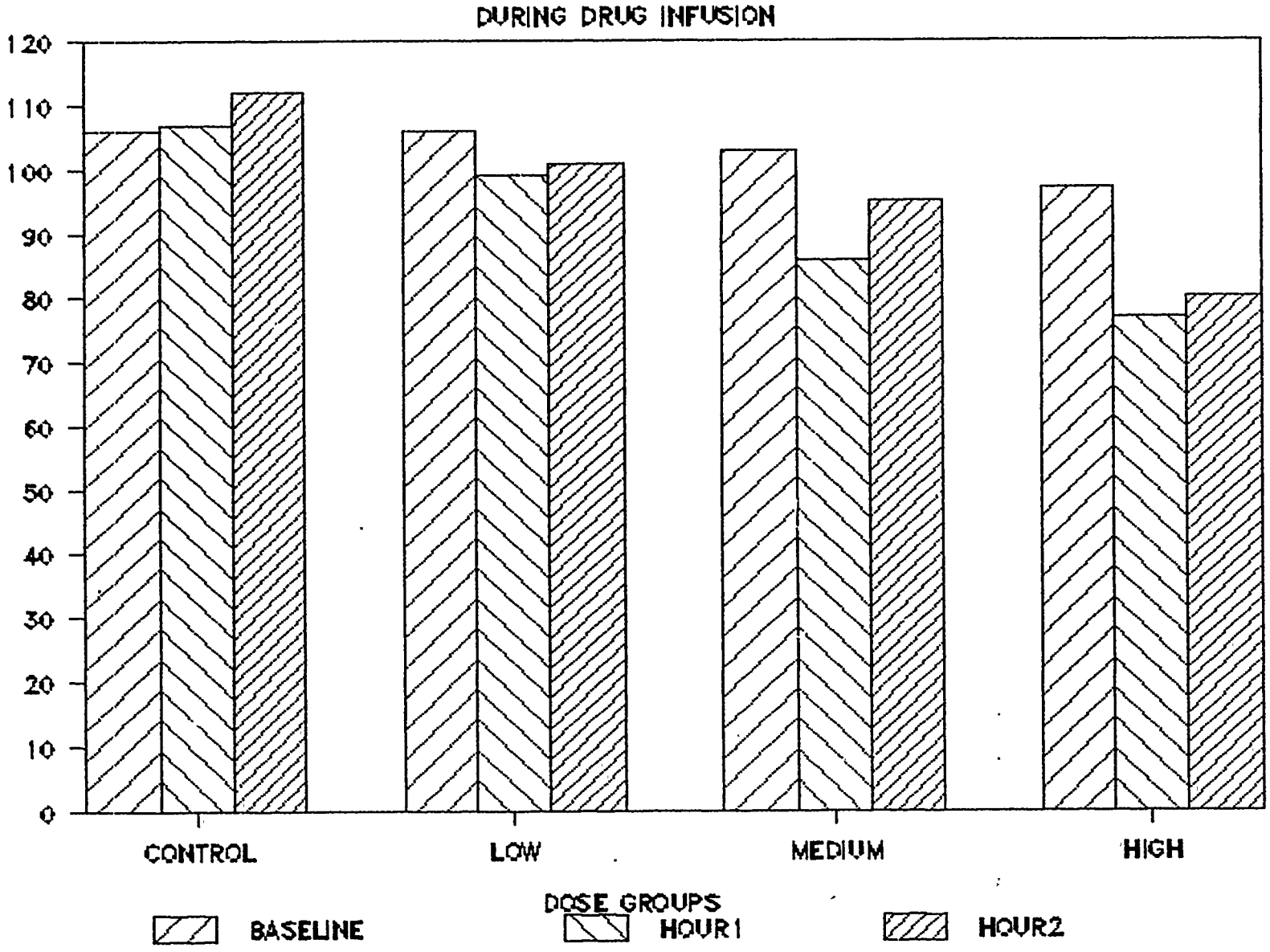
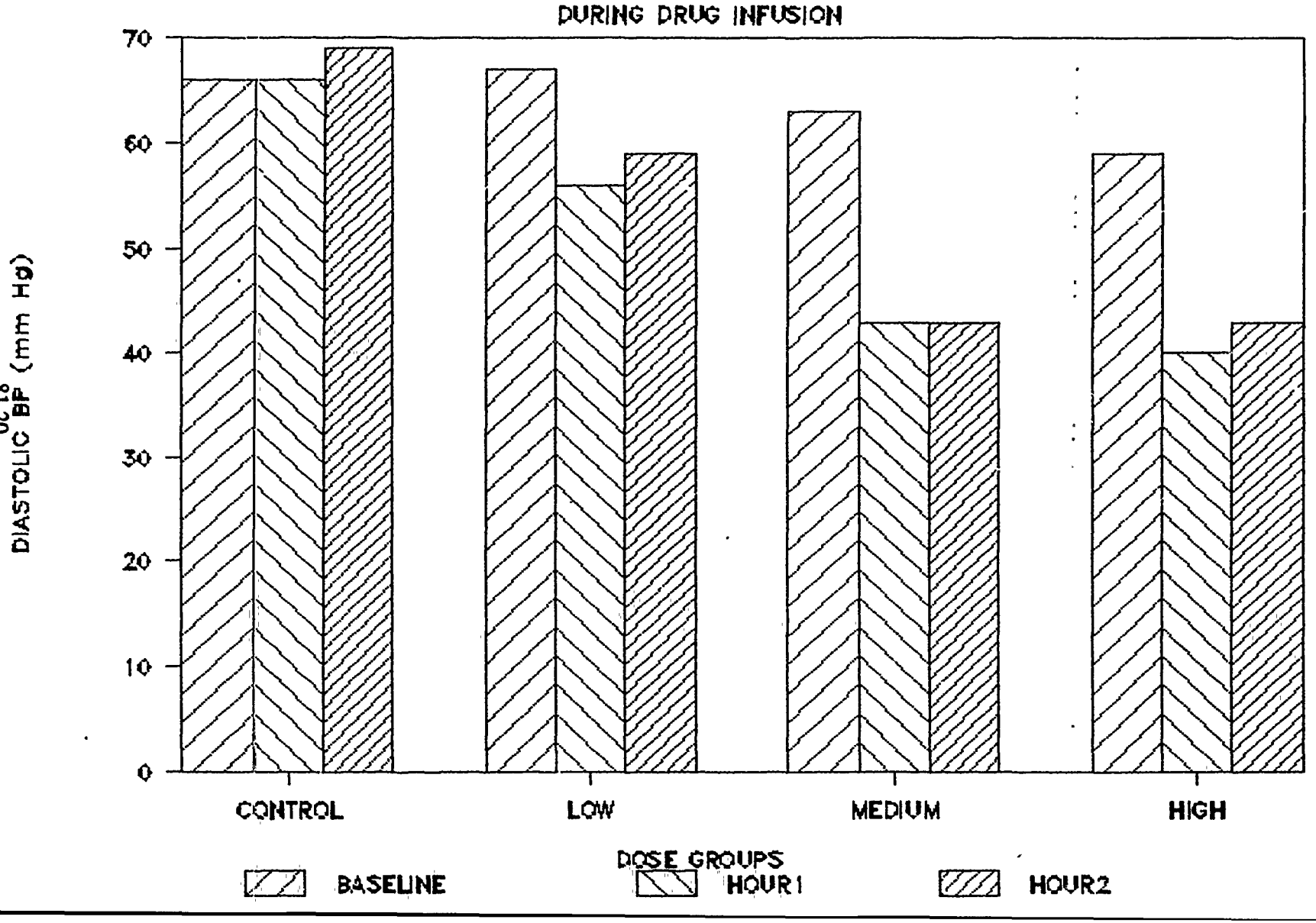


FIGURE 4: AVERAGE DIASTOLIC BP



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FINAL REPORT

Investigation of Selspot II Motion Analysis System Response to Impact Conditions

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Date:	15 September 1989
Contract No:	F49620-88-C-0053

Investigation of Selspot II Motion Analysis System Response to Impact Conditions

by

Laura A. Pytel

ABSTRACT

The Selspot II Motion Analysis System was obtained to test under impact conditions. A pendulum-like motion standard was designed to fit on an impulse accelerator sled. Tests were run to determine the accuracy and integrity of the Selspot II system under acceleration levels up to 24 Gs.

Minor changes were made with the Selspot II cables to avoid a disconnection with the cameras which occurred at the 20 G level. A correction was made on the Selspot II data to account for camera vibration by a rotation of the data plane to fixed targets. The accuracy of the Selspot system's analysis of the pendulum-like motion standard was then determined through comparison to a high resolution potentiometer mounted on the pendulum.

Preliminary results point to the conclusion that the accuracy of the Selspot II system will remain under 1% of the measuring range during accelerations conditions below 21 G's.

Acknowledgments

I would like to thank the Air Force Systems Command and the Air Force Office of Scientific Research for making this research possible through their sponsorship. The administrative and directional assistance from Universal Energy Systems was also very appreciated.

My experience was very rewarding and enjoyable because of many different people. Chris Perry provided me with a place to begin and the freedom to carry out the research under my own direction. He was always available for moral support, friendship, and to help with any problems encountered along the way. Lt. Dena Bonetti was a major benefactor in the early planning and troubleshooting of the project. Manfred Berger of Selspot was a great help in eliminating the bugs of the Selspot system. The entire Biomechanical Protection Branch was a very enjoyable group who provided a pleasant working environment.

A special thanks to DynCorp for their patience and technical support during the test phase of the project and especially to Steve Mosher for his assistance throughout the research effort.

I. INTRODUCTION

The Biomechanical Protection Branch's primary mission is to develop protection technology and protection systems for mechanical stress environments, and to determine the interaction between the dynamic response of the human body and the protection systems within these stress environments.

The laboratory facilities used for research at AAMRL/BBP include a horizontal impulse accelerator and a vertical drop tower.

The motion analysis system is a critical component in the evaluation of the dynamic response of the human body during protection system testing. Currently technicians use 16 millimeter high-speed film cameras to record the motion of marked points of interest on the object being studied. After the film is processed, an x-y film digitization system is used to track the marked points frame by frame, thereby determining their respective displacements and velocities. Because the distance traveled by the subject during the data collection covers a range too wide for a stationary (or off-board) camera to see, the cameras must be mounted on-board and therefore must withstand the same acceleration impact as the test subject. When using this high-speed film method, test results are not received until weeks after testing has been completed.

The Selspot II and MULTILab systems together are a motion analysis and data collection system composed of both hardware and software. Selspot II will collect, process and present motion data all under the control of the MULTILab software. Throughout the remainder of this report, the Selspot II/MULTILab motion analysis system will be referred to as the Selspot system. An upgrade of the present motion analysis system

would save the branch over 80,000 dollars a year while providing results in less than an hour.

However, the Selspot system's characteristics have been verified only when the cameras are stationary. This introduces the need for testing of the Selspot system under impact conditions.

As a mechanical engineer, my background includes vibration theory, electrical instrumentation and mechanical design. My previous research work involving gravity leveling instruments is also quite complimentary to the testing of the Selspot system.

II. OBJECTIVES OF THE RESEARCH EFFORT

Since the current motion analysis techniques are slow and expensive, possibilities for a new motion analysis system were examined. To update the system with a newer version high-speed film system would be extremely expensive. Therefore the possibility of upgrading to the Selspot system was investigated.

The main objective of this research effort was to determine the 'usefulness' of Selspot as the motion analysis system for human testing conducted on the horizontal impulse accelerator sled and the vertical drop tower of the AAMRL/BBP test facility.

Being a 'useful' motion analysis method requires the following characteristics of the system in an impact environment of up to the 12 G acceleration level for human testing:

1. Accuracy level must remain within 1% of the measuring range.
2. Resolution must be less than 1% of the measuring range.
3. The integrity of the system must be maintained through repeated impacts.
4. The targets must be able to adhere to the subject without falling off or restraining the movement of the test subject.
5. The system must be able to make 3-D transformations as well as compute displacements, velocities, and accelerations of the individual targets.
6. The sample rate of the system must be greater than or equal to 350 Hz for up to 10 targets.
7. The targets must still be visible even when they point 45 degrees away from the cameras.

A secondary objective added to this research project was to determine

the accuracy of an angular accelerometer.

My assignment as participant in the 1989 Graduate Student Research Program (GSRP) was to design, conduct and conclude a test which would meet these objectives.

III. APPROACHES TAKEN TO MEET OBJECTIVES

The Selspot system has been used in impact testing by automobile companies such as Chrysler, Volvo, and Renault as an off-board motion analysis system for about five years. Knowing this, Selspot was assumed to be functional to the specifications listed by the Selspot company (division of Selective Electronics, Inc.) as an off-board system. Therefore, the Selspot system already meets all of the requirements listed in Section II, except for those characteristics which are affected by an impact.

Many of the requirements that are not affected by impact, such as sample rates, target visibility, and software capabilities, are already met by the system. However, rough checks of these stationary requirements were conducted on the Selspot before any impact tests were scheduled. The remaining requirements of accuracy, structural integrity and target attachment must therefore be tested under impact conditions before the Selspot system can be accepted.

The angular accelerometer must also be tested during impact conditions to examine it's purely angular motion analysis..

IV. TEST PLANS

A research effort was planned to determine the accuracy and integrity of the Selspot system under impact conditions. A method for effective target attachment was devised during impact testing as well.

The new system was only desired for human testing which occurs at 12 G impacts or less; therefore, the test matrix (shown below in table 1), went to 24 G allowing a safety factor of 2.

TABLE 1

ACCELERATION EXPOSURE (G)	PULSE DURATION (MS)	TEST CELL	NUMBER OF TRIALS
0	-	A	3
6	160	B	3
10	159	C	3
15	159	D	3
20	143	E	3
24	126	F	3

To determine the accuracy of the Selspot system during impact, a motion standard was developed. This consisted of a pendulum-like device with 3 targets each mounted at a different distance from the pivot point.

Two Selspot cameras analyzed the motion of the targets after impact. Since the cameras were viewing the targets from different angles, the

actual 3-D coordinates of motion could be calculated. From the 3-D coordinates, the displacements were found at each given time interval for each target. These displacement values were then compared to 'accepted' values for displacement found by a high resolution potentiometer mounted at the pendulum pivot. Finally, the error in the accuracy of the Selspot system was defined as the difference between the Selspot value and the potentiometer value. It should be noted that the moving targets were corrected for camera vibration before the error analysis was calculated. This was done by analyzing the motion of four fixed targets, then rotating the camera position such that the fixed targets appeared stationary.

The integrity of the system was measured simply by visual inspections of the different components as well as watching for drastic changes in the accuracy of data or any other functions of the hardware.

Since the pendulum-like motion standard created angular acceleration, an angular accelerometer was mounted on the pendulum, three inches from the pivot point. This was an additional accuracy test of motion analysis. The angular accelerometer output was integrated twice to get displacement then compared to the accepted displacement value from the potentiometer. Again, the difference between the two displacements was taken as the error of the angular accelerometer.

V. TEST RESULTS

Testing was conducted without incident until the 20 G level was reached. The second test at this level showed that one of the LED's had flashed in place of two others. This problem became common at the 20 G level. After several static tests, the problem was pinpointed to a disconnection occurring at the camera/multi-cable connection which was rather weak. This was greatly improved by cleaning the connector and wiring the cables such that only one cable went to each camera. Any further problems with this error can be corrected using software which will interpolate over data points of extreme displacement.

When the acceleration level was raised to 24 G's, the cameras and housings were too heavy for the mounts to hold. Both mounts were bent and loose. The cameras had to be braced with additional supports. It was decided that the 24 G level tests were not crucial and therefore were dropped from the test schedule. More tests were then done at the 20 G level to test for the disconnection problem. The problem still occurred but was less frequent.

The data analysis consisted of first correcting the Selspot data for camera vibration. The displacement data from the Selspot and the angular accelerometer were compared to the potentiometer displacement data. The error was defined as the difference between the Selspot (or angular accelerometer) and the potentiometer displacement data. This difference was found every two milliseconds for the first 100 milliseconds following impact. The mean error for each G level was tested to see if it was under the criterion value of 1% of the measuring range which is equal to 0.55 inches.

At this time, the accuracy information has not been completed. However, preliminary results are very promising. The following plots show the most current results from this test.

Figure 1 shows the view of the 7 LED's as seen by the Selspot system before vibration correction for a 6 G test. Led's no. 4, 5, 6, and 7 are all stationary on the sled, while led's no. 1, 2, and 3 are mounted on the pendulum. This becomes more clear when the data have been corrected for camera vibration. The display in figure 2 represents the same data after manipulation by the camera vibration correction software. The difference between the Selspot displacement data and the potentiometer displacement data for this test is shown in figure 3. Figure 4 shows the comparison between the displacement seen by the potentiometer overlaying the displacement recorded by the angular accelerometer. Rough estimates of overall accuracy made from figures 3 and 4 show favorable results for both the Selspot and the angular accelerometer.

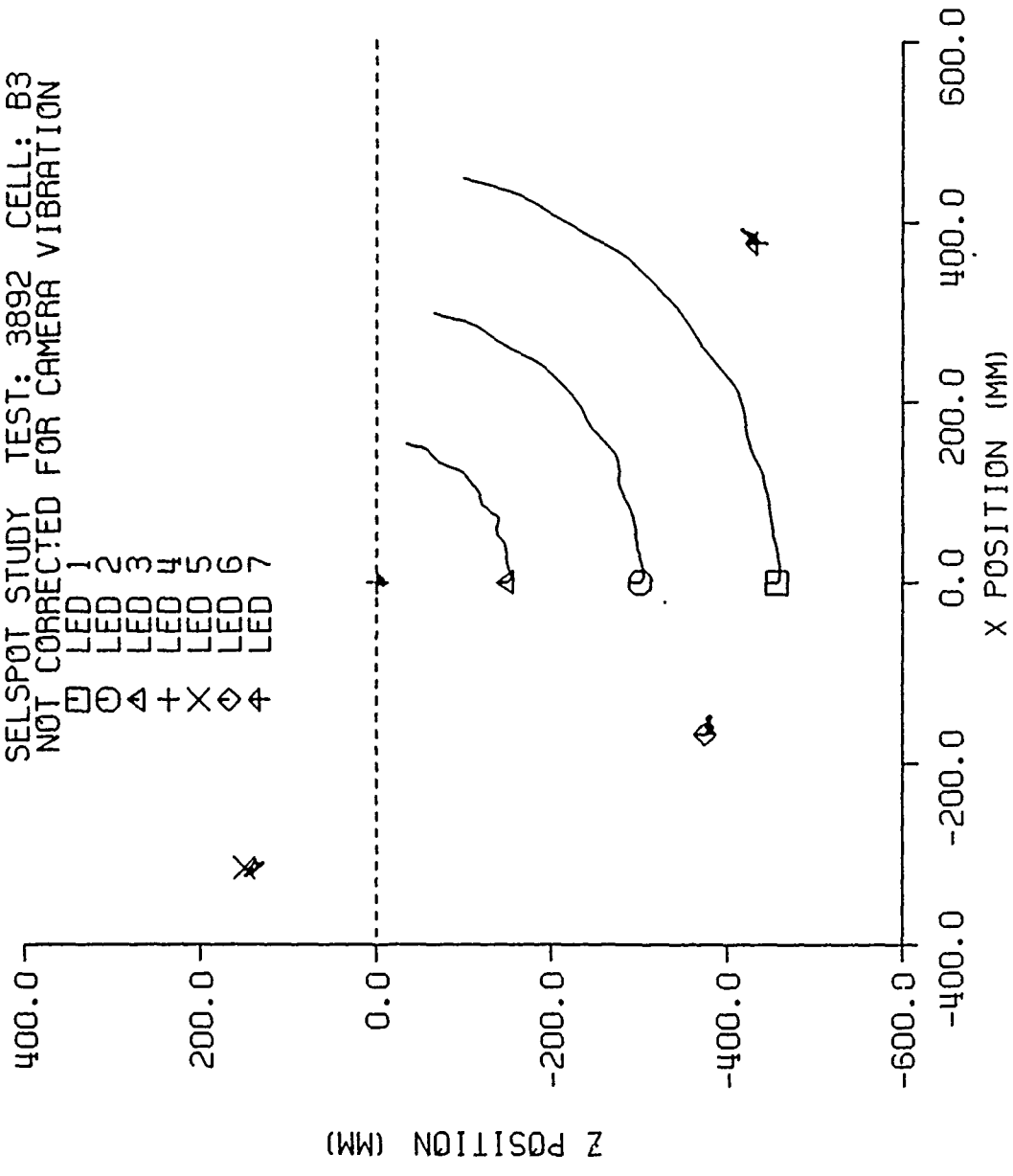
SELSPOT STUDY TEST: 3892 CELL: B3
NOT CORRECTED FOR CAMERA VIBRATION
LED 1
LED 2
LED 3
LED 4
LED 5
LED 6
LED 7
400.0
200.0
0.0
-200.0
-400.0
-600.0
Z POSITION (MM)
-400.0
-200.0
0.0
200.0
400.0
600.0
X POSITION (MM)

FIGURE 1

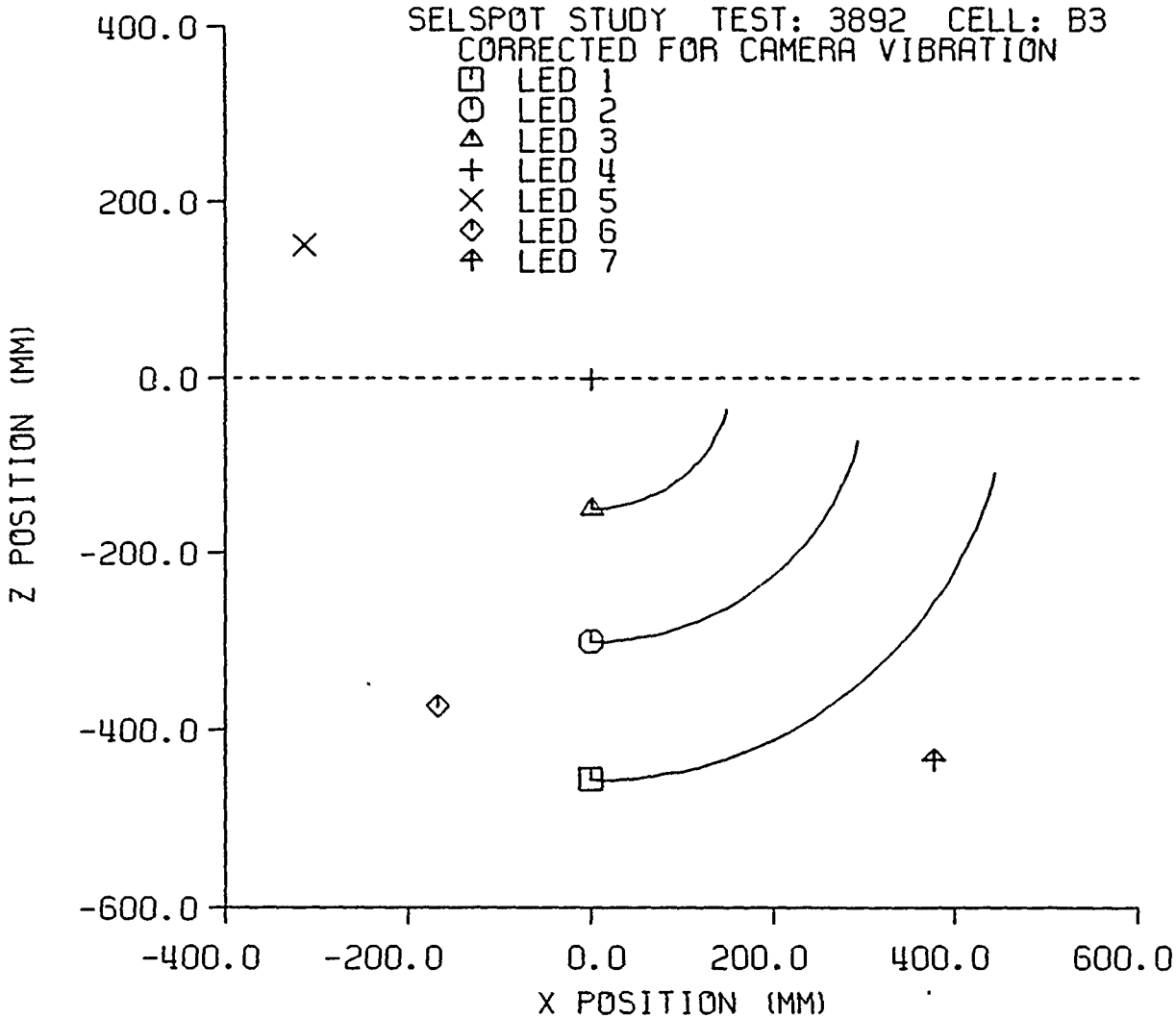
SELSPOT STUDY TEST: 3892 CELL: B3
CORRECTED FOR CAMERA VIBRATION
LED 1
LED 2
LED 3
LED 4
LED 5
LED 6
LED 7
400.0
200.0
0.0
-200.0
-400.0
-600.0
Z POSITION (MM)
-400.0
-200.0
0.0
200.0
400.0
600.0
X POSITION (MM)

FIGURE 2

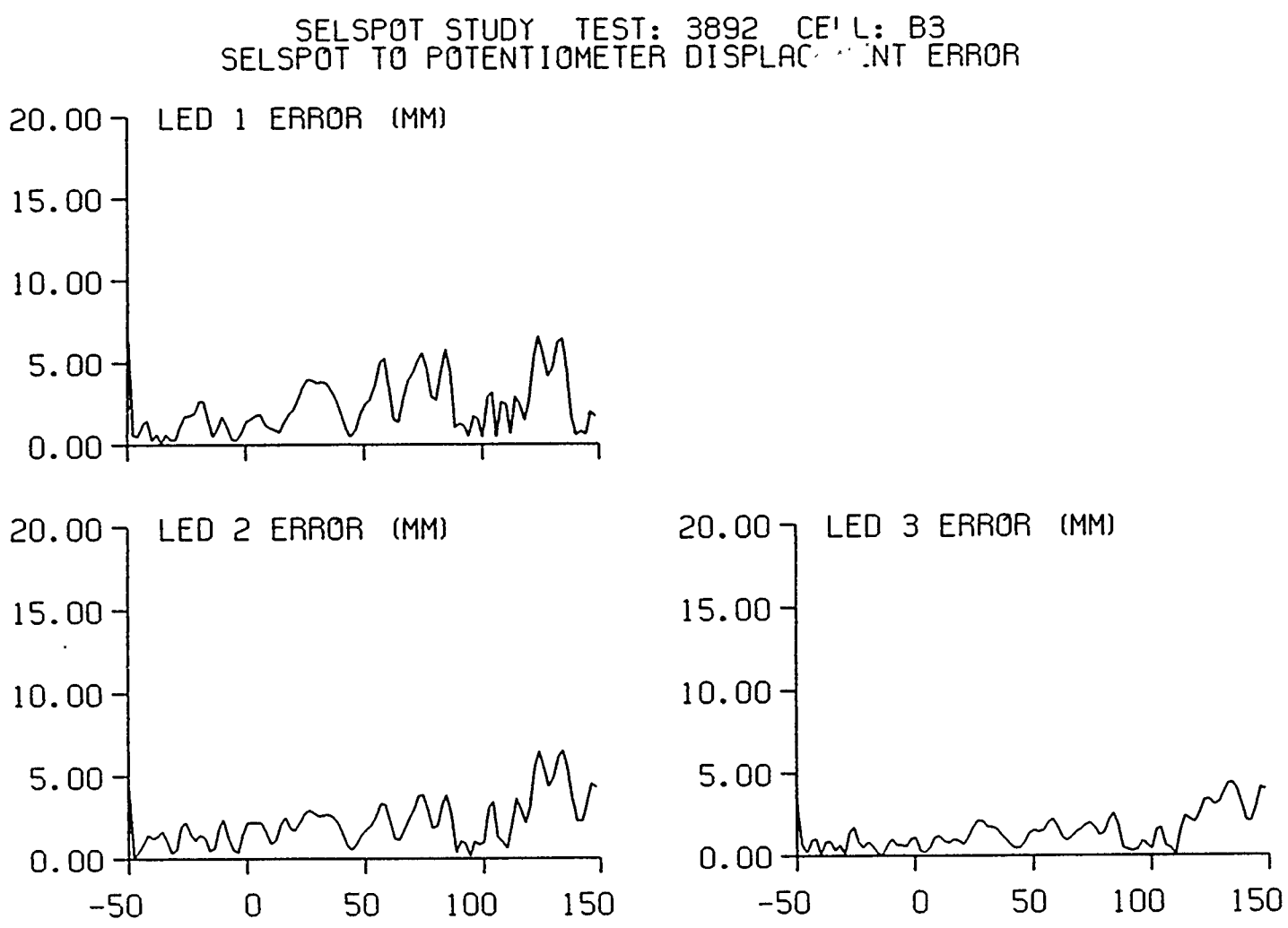
SELSPOT STUDY TEST: 3892 CELL: B3
SELSPOT TO POTENTIOMETER DISPLACEMENT ERROR
LED 1 ERROR (MM)
20.00
15.00
10.00
5.00
0.00
LED 2 ERROR (MM)
20.00
15.00
10.00
5.00
0.00
-50
0
50
100
150
LED 3 ERROR (MM)
20.00
15.00
10.00
5.00
0.00
-50
0
50
100
150

FIGURE 3

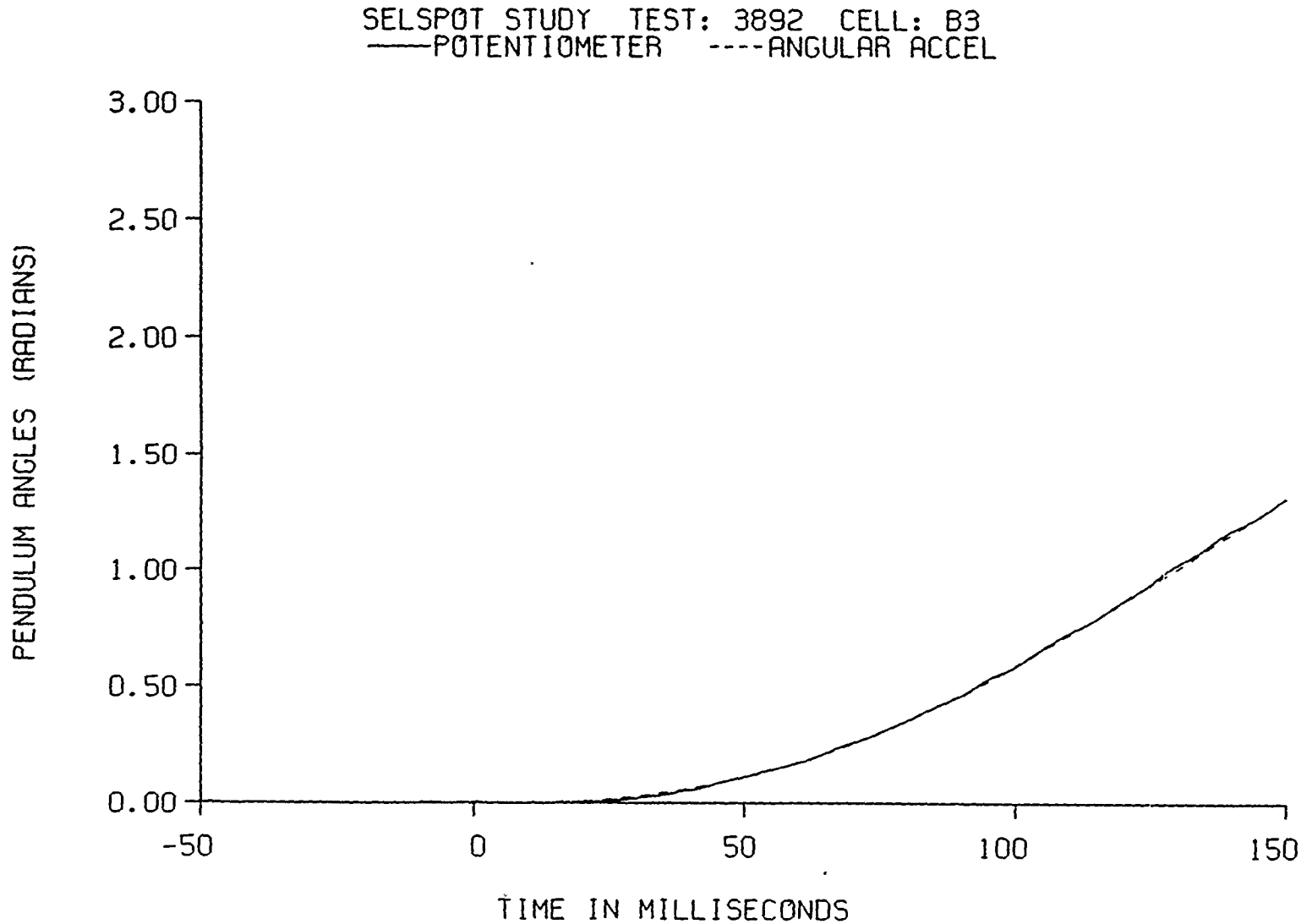
SELSPOT STUDY TEST: 3892 CELL: B3
——POTENTIOMETER ----ANGULAR ACCEL
PENDULUM ANGLES (RADIANS)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-50
0
50
100
150
TIME IN MILLISECONDS
82-16

FIGURE 4

VI. RECOMMENDATIONS

This research has concluded that the Selspot system would be very beneficial as a motion analysis system for use in research conducted by BBP on the horizontal accelerator sled and the vertical drop tower at a maximum acceleration level of 20 G's. This is assuming that the accuracy checks out to be less than 1% of the measuring range.

Some recommendations for implementing the Selspot system are as follows:

1. The calibration procedure should be carried out at the beginning of each day of testing. By dating the files, the correct transformation data base can be matched to the corresponding data files taken the same day.

2. The calibration could be greatly simplified by placing the fixed targets at known locations in the seat coordinate system. This would eliminate the need for the calibration position reference standard (PRS) and the data from another stationary position.

3. The camera housings could be lightened by about 30% if some redundant sections were removed. This would be necessary for tests over 15 G.

4. The camera mounts and supports need to be redesigned for increased strength in the direction of sled movement. The I-beam section of the support should be used in the axis parallel to the sled movement. The mounts need to have more than one bolt holding them to the support. Two bolts could be placed in separate quarter circle slots to allow the same rotation as the present mount. Also a bolt should be placed in the middle of each side in addition to those at each corner.

5. Elastic armbands or tape could be used to keep the LED (target) leads in place. The LEDs themselves can be attached with double back sticky foam pads.

6. At least five of the LEDs with long length (2.5 m) leads should be purchased. These would be used for points at a large distance from the LCU box.

7. A removable insulator should be placed around the LCU box to enclose LED connections which remain at 20 volts.

8. All seat fixtures and surrounding supports etc. which are in the view of the Selspot cameras during testing, should be painted flat black if possible.

9. A label should be placed on the back of the two cameras to remind technicians to turn off power before disconnecting or connecting the cameras. This will avoid possible damages to cameras and LEDs.

10. A vibration correction program should be written on the Selspot system to avoid transferring data to the VAX.

11. A correction program should be implemented that looks out for extreme data, removes it, and interpolates between the adjacent points.

12. A cassette storage system should be purchased from Selspot to permanently store the test data more easily than on circular reels. This along with the vibration correction would completely eliminate the need for transferring data to the VAX.

13. The actual principal distance for a 24 millimeter lens should be calculated by Selspot and the software adjusted accordingly.

14. Compatibility of the Selspot/MULTILab software to standard languages such as Fortran or Pascal by Selspot would improve the usefulness of the system.

15. (optional) A plotter should be purchased which is compatible to the plotter port of the Selspot system for displaying test results.

16. (optional) A larger lens (shorter focal length) could be purchased as a standby lens, or for wider views. All lenses should have compatible connectors as well as the same size support from the housing.

17. (optional) An A/D board can be purchased from Selspot to insert into the central control unit of the Selspot system which could handle up to 64 analog channels without affecting the speed of the LED (target) sample rate.

To continue the investigation of the Selspot system, tests could be conducted at higher G levels using stronger mounts and supports. Further research into the Selspot's use on the drop tower could also reveal whether the water spray created after impact would impair the LEDs in some way.

However, within the recommendations listed above, the Selspot should prove to be a valuable motion analysis system for both the horizontal accelerator sled and the vertical drop tower at impact levels below 21 G for AAMRL/BBP research in the near future.

If the Selspot system's accuracy does not prove to be within 1% of the total measuring range, further testing would be required before the system could be accepted unless the requirement is changed. The further testing should take place entirely within the Selspot system. An A/D board should be obtained to synchronize the timing of both the potentiometer and the Selspot system for a more accurate comparison.

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FINAL REPORT

SPEECH CODING AND FEATURE RECOGNITION WITH A BACKPROPAGATION NEURAL NETWORK

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USAF Researcher: Mr. Timothy R. Anderson

Date: 17 Sept 89

Contract No.: F49620-88-C-0053

Speech Coding and Feature Recognition with a Backpropagation Neural Network

by
Janet Slifka

ABSTRACT

The backprogation (bpn) neural network was investigated in relation to speech coding through the use of an identity mapping network. Training a 50-20-50 network produced a mean square error on the order of 0.01. From this it was concluded that all essential speech information was compressed to 20 points from 50 points. These compressed points were fed into another bpn network whose goal was to determine if the data presented was a vowel, consonant or silence. Preliminary testing in this area was conducted, producing several areas for further research.

Acknowledgements

The author would like to thank the Armstrong Areospace Medical Research Laboratry as well as the Air Force Office of Scientific Reserach for the opportunity to conduct this research. Also, Universal Energy Systems deserves gratitude for their particiption in this effort.

This research experience has been very beneficial to me as a result of the high caliber of researchers that I was able to work with. I would especially like to thank Tim Anderson for his continual support and ready information. I would also like to thank Dr. Nixon for his open attitude and obvious enjoyment in his work.

1. INTRODUCTION

Neural networks, a growing area in the world of engineering, offer as one of their most attractive features the ability to execute a mapping without extensive and exhaustive statistical analysis of the data involved. This is especially attractive to the area of speech because this type of data can vary enormously depending on speaker, tone of voice, accent, pronunciation and other factors.

The Biological Acoustics Branch of the Armstrong Aerospace Medical Reserach Laboratory (AAMRL) at Wright Patterson Air Force Base is conducting basic research in speech recognition, focusing on several areas. The particular research presented within this report focuses on speech coding to reduce the number of data points required in a speech recognition system. Speech data is well known for its large data storage requirements and any reduction in this area could speed the analytial process. The second area pertained to preliminary studies in feature recognition. The desired goal is a network that could recognize each possible phoneme from any given speaker. Toward that end, research was conducted on a network that could classify data into one of three categories - vowel, consonant, or silence.

My research history has been focused on digital signal processing techniques as related to biological signal processing. In the past, this has been concentrated on the detection and estimation of evoked brain potentials using an adaptive filter. This experience and my desire to expand my area of research from the single layer system to a multi-layer network contributed to my assignment to the Biological Acoustics Branch at AAMRL.

2. OBJECTIVES OF THE RESEARCH EFFORT

The 50-20-50 identity-mapping backpropagation neural network, had been previously implemented within the laboratory by Mr. Tim Anderson. This was accomplished on a Symbolics system with a high-level neural network software package. This software allows realtively easy set-up of various network configurations. The Biological Acoustics Branch also has within its possesion, a Hecht-Nielson (HNC) Neurocomputer which is capable of many of the same functions as the mentioned software package but requires more detailed and application-specific programming. The extra time involved in this programming gains a considerable advantage in processing time. Time for utterance processing and network

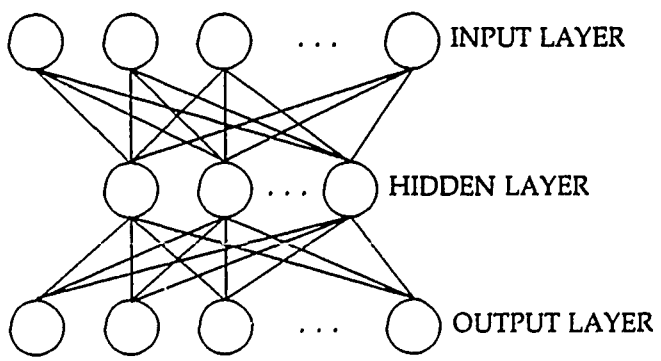
training is cut by approximately a factor of sixteen.

It was my assignment to develop the capabilites of the neurocomputer within the areas indicted to be promising on the Symbolics system. This involved implementation, in 'c' programming language, of the identity map and corresponding screen and disk outputs. Further research was conducted to use the hidden layer of the network as coded input to a feature classification network.

3. BACKGROUND

A neural network consists of a number of processing elements (PEs) or units, their various connections to the other PEs, the learning laws that govern the training and adaptation of the PE weights, and the data used to train the network. For the networks within this research effort, the configuration in Figure 1 was used. This is a three-layer network consisting of an input slab, hidden slab, and output slab. A connection exists between each input PE and each hidden unit and between each hidden PE and each output unit. Each unit has an activation level. Usually, the activations of the input layer are simply the input data. For the input slab, no calculations are performed to determine the activation level. However, for the hidden and output slabs, the activations are determined by a weighted sum calculation. Each unit has a weight associated with each connection. The activation is computed as the sum of each weight value multiplied by the activation level on the connected PE. Each unit also has an input of one that is included in the weighted sum after being multiplied by a bias weight.

Figure 1 : Network Configuration



A circle represents a PE and a line represents an interconnection.

Neural networks provide a means for extracting information from data. This takes the form of mapping a set of input data points into a set of output points. Usually this involves some sort of classification process as will be discussed in the vowel/consonant/silence (v/c/s) classifier. A special case is the identity mapping neural network in which the network attempts to reproduce the input data. This serves to 'load' the hidden layer of the network with all of the information in the input layer. If the number of units in the hidden slab is less than the number of input units, a data compression has been achieved.

The network is trained by adapting the slab weights to the desired mapping. The updating of the weights is governed by a learning law. For the applications considered here, the backpropagation law is used. The network is presented with the desired output for the current set of inputs. The weights are updated so as to minimize the mean square error (MSE) of the output units in relation to the desired output or training data.

Several defintions are necessary to detail the learning law and adaptation process.

w_{ij} : *an adaptive hidden slab weight.*

u_{ij} : *an adaptive output slab weight.*

Δw_{ij} : *the change in the hidden slab weight.*

Δu_{ij} : *the change in the output slab weight.*

o_i : *the activation of the ith output PE.*

h_i : *the activation of the ith hidden PE.*

d_j : *the activation of the jth input PE.*

t_i : *the training input.*

α_o : *the learning constant.*

β_o : *momentum constant.*

$f'(s_i)$: *the deriviative of the activation function.*

These are used to define the learning law for the output slab as follows:

$$\delta_{oi} = f'(s_i)(t_i - o_i)$$

$$\Delta u_{ij} = \alpha_o \, \delta_{oi} \, h_j$$

$$u_{ij}^{new} = u_{ij}^{old} + \Delta u_{ij}$$

$$f'(s_i) = o_i(1 - o_i) \qquad \text{for } \textit{sigmoidal activation.}$$

For the hidden slab, a slightly different lerning law is applied. In this case, the contribution of each hidden unit to the total error at the output is computed. This should allow the weights to converge to the minimum MSE mapping. This law is defined as follows:

$$\delta_{hi} = [\, h_i(1 - h_i) \,] \sum_{k=1}^{O} \delta_{ok} u_{ki}$$

$$\Delta w_{ij} = \alpha_h \delta_{hi} d_j$$

$$w_{ij}^{new} = w_{ij}^{old} + \Delta w_{ij}$$

where O is the number of processing units in the output layer. With these two learning laws, the backpropagation neural network should proceed toward the desired mapping. However, this is not always the case, especially for higher learning rates. For this reason it is necessary to introduce the concept of momentum. This is a device which allows the convergence to be directed to a general direction. This introduces a modification to the learning rule:

$$\Delta u_{ij}^{new} = \alpha_o \delta_{oi} h_j + \beta_o \Delta u_{ij}^{old}$$

$$u_{ij}^{new} = u_{ij}^{old} + \Delta u_{ij}^{new}$$

$$\Delta w_{ij}^{new} = \alpha_h \delta_{hi} d_j + \beta_h \Delta w_{ij}^{old}$$

$$w_{ij}^{new} = w_{ij}^{old} + \Delta w_{ij}^{new}.$$

This says that the amount that the current weight will be updated is governed by

some proportion of the previous weight change [1,2].

4. DATA

Speech data was obtained from the CD_ROM Texas Instruments (TI)/Massachusets Institue of Technology (MIT) speech database. This is an acousto-phonetic database consisting of 420 speakers with each speaker having 10 sentences. The speakers have been divied into eight dialect regions (dr) designated as follows:

dr1: New England
dr2: Northern
dr3: North Midland
dr4: South Midland
dr5: Southern
dr6: New York City
dr7: Western
dr8: Army Brat (moved around).

Dialect region one has been used in this application. This was an arbitrary choice and the goal was simply to limit the data to a single dialect region for this early testing phase. Utterances have also been divided into several types. The kind used in this research are denoted by the letters 'sx' followed by the sentence number. The 'sx' represents an MIT phonetically compact sentence, of which there are five per speaker. Each utterance is also identified by a set of initials. The set, 'mreb0' represents a male (m) speaker with intials 'reb'. The trailing '0' means that within this dialect region, there is only one speaker with the initials 'reb'. Again, an arbitrary decision was made to use the speaker, 'mreb0', to limit data to a single speaker. This presents a simple data set to the network. Plans do exist, however, to expand training and testing to several speakers and dialect regions.

The utterances are recorded at a sampling rate of 16 kHz with a header and trailer of silence. Each utterance was divided into 50-point records with a maximum of 1000 records for processing, depending on the length of the particular utterance. Each utterance has associated with it, three files -- the actual utterance data samples, a text version of the utterance, and a phonetic breakdown of the utterance. These are useful in analyzing the data.

5. IDENTITY MAPPING NEURAL NETWORK

The network itself was required to have the ability to be trained on a set of speech data as well as test a trained set of weights. Pertinent variables in the backpropagation network configuration are listed below. These were determined from prior research on the Symbolics system.

- Sigmoid decision function.
- No direct connection from input PEs to output PEs.
- Momentum weight updating.
- Hidden alpha and output alpha of 0.2
- Hidden momentum (beta) and output momentum of 0.9.

All data was normalized internal to the program before input to the network. Before data is stored to the disk it is rescaled. However, the stored weights apply to the normalized data.

A primary goal of this research effort was to develop an easily-used software package on the HNC Neurocomputer for analysis of the two mentioned networks. Toward this goal, the developed software has several capabilities. For data access, data can be read directly from the CD_ROM TI/MIT database. This binary data is converted to ASCII format for internal use.

Data can be loaded either sequentially (as in for testing or training a saved set of weights) or randomly choosing 50-point data sets from throughout the utterance (as in for training a network). Analysis can be done in the single-utterance sequential or random mode. Or, with the capability to change data files at any time, analysis can be done in a 'piggyback' sense. The network may be trained on one data set a number of times and then trained on a different uttrance data set an additional number of iterations, etc. Also, the capability exists to randomly access up to four different utterances simultaneously and extract a random data record each time.

For evaluation purposes, the display screen presents a real-time plot of both the input and output waveforms of the identity map, the actual phoneme in use and its actual and predicted v/c/s classification, the number of training/testing iterations completed, and the current MSE and Mean Actual Error (MAE), where MSE and MAE are defined as:

$$MSE = \frac{1}{N}\sum_{i=1}^{N}\sum_{j=1}^{O}(t_i - o_{ij})^2$$

$$MAE = \frac{1}{N}\sum_{i=1}^{N}\sum_{j=1}^{O} | t_i - o_{ij} | .$$

Options exist to switch screens to a display of a real-time plot of the MSE for both the identity map and the v/c/s calssifier and a listing of v/c/s recognition statistics including percent correct for each possible type and total number of correct predictions. On another screen the current activation level for every PE in the network system can be displayed. Elapsed training time can be displayed and the network can be switched between training and testing at any time. Also, the current network weights can be saved on disk for future use and the learning law parameters of alpha and beta can be changed at any time. These various analytical tools allows the network to be evaluated in several different aspects. Figures 2 and 3 show typical screen displays. Figure 2 shows the main display screen for the identity mapping data. This network uses the weights saved after training sequentially 1 million times with the utterance sx205. Figure 3 shows the MSE plots for a network system beginning the training process. The large MSE at the start is due to randomly initialized weights. This decreases as the networks learn the mapping for the header of silence. When the actual utterance begins, there is a sharp inrease in MSE as the networks adapt to the presentation of vowel consonant data.

In the test mode, each record is iterated once through the network and the entire processed utterance is then stored to disk. In the training mode, no processed utterances are stored and as many iterations as desired can be executed. The records, for training, are presented to the network in a 'ring' fashion, that is, 1000 records of training data would be presented in the same order repeatedly until training is completed.

6. TEST RESULTS

Data used in early testing is from Dialect Region 1, Speaker mreb0, utterances sx115(806 records), sx205(972 records), sx25(696 records), and sx295(1000 records). These utterances are as follows:

sx25 : Only lawyers love millionaires.

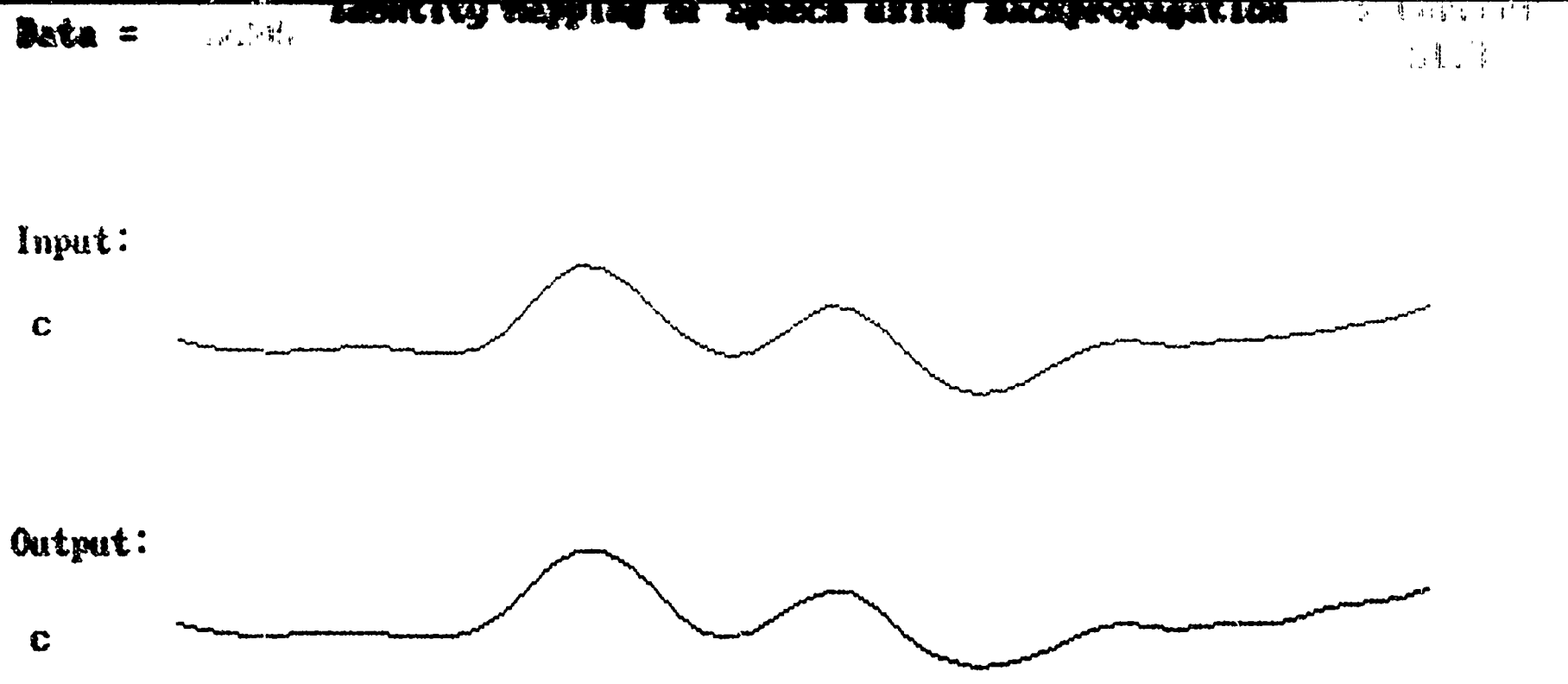


Figure 2: Identity Mapping Display Screen

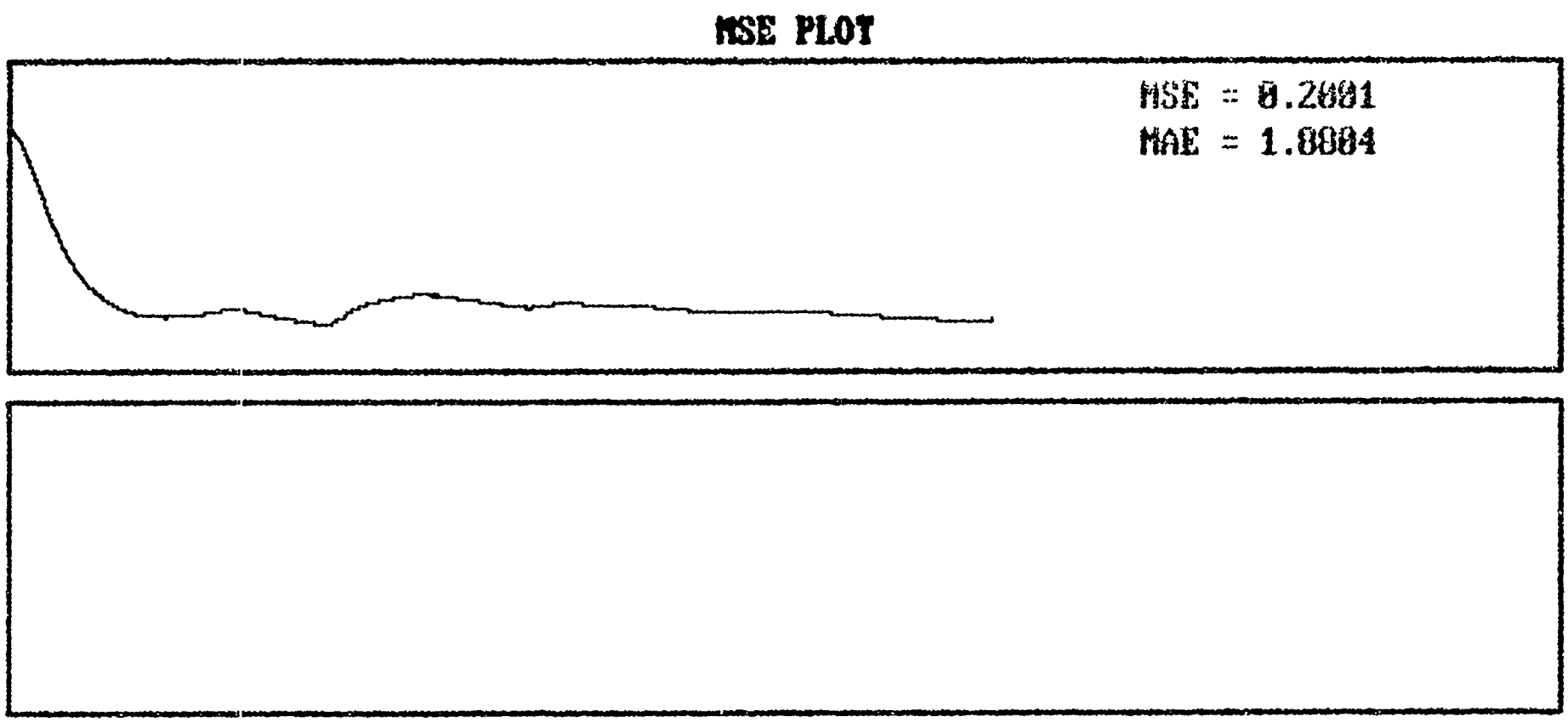


Figure 3 : MSE Plots and v/c/s Classification Statistics

sx115 : The emblem depicts the Acropolis all aglow.

sx205 : Military personnel are expected to obey government orders.

sx295 : If Carol comes tomorrow, have her arrange for a meeting at two.

Training

Net #	Utterance	# of iterations	Type of training	MSE
1	sx115	100,000	sequential	0.0259
2	sx115	100,000	random	0.0273
3	sx115 + sx205	100,000 ea.	piggyback, random	0.0157
4	sx115,sx205,sx25 and sx295	100,000	random file selection and random record selection	0.0252

The third entry, sx115 + sx205, is the only set of training to use 200,000 iterations total. This may make comparison with the other trained networks unfair. The additional training time could noticeably reduce the mean square error. Training 100,000 iterations takes approximately fifteen minutes.

Testing

Utterance	Trained Net #	MSE
sx115	1	0.0171
sx115	2	0.0201
sx115	3	0.3946
sx115	4	0.0991
sx205	1	0.7139
sx205	2	0.7012
sx205	3	0.0201
sx205	4	0.3108
sx25	2	0.1694
sx25	3	0.1362
sx25	4	0.0364
sx295	2	0.2491

sx295	3	0.0728
sx295	4	0.0505

Network number four uses 696 test records and samples randomly from each of the four listed files. This means that the network sees the data from each file for less than one-fourth the time that the other networks do. Taking this into account, this network performed remarkably well.

It appears that piggyback training degrades recognition of the first training data faster than the random file selection training. It would be interesting to train the random file selection network 200,000 times and then compare with network #3.

It was observed that the utterance sx205 had more high frequency sinusoid-type data sections, associated with the phonemes 'z' and 's', than the other tested utterances. This might contribute to the high MSE found when testing sx205 on nets 1, 2, and 4.

5. FEATURE EXTRACTION

The identity mapping had the advantage of previous research to determine network parameters. The v/c/s identifier network did not have any previous research. For this reason, the research is basic and attempts to determine which set of variables allow the 'best' identifier to emerge. Due to coding problems with the joined network configuration, run-time is relatively high. As of this writing, the problem has not been solved. In an attempt to present at least preliminary results, testing has been conducted in a limited sense. The time invloved did not allow for complete training of the network.

The test setup involved using the hidden layer of the identity-mapping network as the input to the v/c/s classifier. The two output units of the network were designted as follows: 0.1, 0.1 for silence, 0.1, 0.9 for consonant, and 0.9, 0.9 for vowel. This binary-type representation used 0.1 and 0.9 to decrease the distance each activation had to switch to designate another type. This was necessary due to the fact that most error is introduced in the delay present when switching between phoneme types. Also, an extra set of connections were added. The input slab was connected directly to the output slab.

When evaluating the performance of the v/c/s classifer, it is important to consider

an untrained network. A random set of weights will produce a single guess as to the phoneme type. If the network were to guess 'vowel' and the vowel/consonant distribution in the utterance was approximately 50-50 with the silence portion being almost negligible, then the network would be correct 50% of the time. In order to justify using the network, recognition should exceed that possible through a 'guess' and should show recognition of each type - vowel, consonant, and silence.

The identity-mapping network was trained 1 million iterations with the sx205 utterance in a sequential manner according to the run-time options mentioned above. Sequential training was chosen over random because it gave an almost even representation of vowel and consonant samples and could be repeated with exactness. Two statistical measures were taken. These were MSE, the average squared error value over all iterations and MAE the average actual error over all iterations. Training produced an MSE of 0.0118 and a MAE of 0.3386. Testing the sx205 utterance on this trained network produced an MSE of 0.0057 and an MAE of 0.3155.

Any v/c/s network output over 0.5 was designated a '1' and any below 0.5 was designated a '0'. This allowed for compliation of classification percentages.

Preliminary Testing

8 Hidden Units, 5000 Training Iterations
Alpha 0.3, Beta 0.8
MSE: 0.1795 MAE: 0.3452

	% Correct	Total Correct	Total Seen
V	0.5	2	404
C	100.0	481	481
S	0.0	0	86
T	49.7	483	971

8 Hidden Units, 5000 Training Iterations
Alpha 0.2, Beta 0.9
MSE: 0.2249 MAE: 0.4224

	% Correct	Total Correct	Total Seen
V	0.0	0	404

C	100.0	481	481
S	0.0	0	86
T	49.5	481	971

10 Hidden Units, 5000 Training Iterations

Alpha 0.3, Beta 0.8

MSE: 0.2168 MAE: 0.4517

	% Correct	Total Correct	Total Seen
V	0.5	2	404
C	100.0	481	481
S	0.0	0	86
T	49.7	483	971

10 Hidden Units, 5000 Training Iterations

Alpha 0.2, Beta 0.9

MSE: 0.2386 MAE: 0.4382

	% Correct	Total Correct	Total Seen
V	0.0	0	404
C	100.0	481	481
S	0.0	0	86
T	49.5	481	971

8 Hidden Units, 520,000 Training Iterations

Alpha 0.2, Beta 0.9

MSE: 0.2861 MAE: 0.5694

	% Correct	Total Correct	Total Seen
V	0.0	0	404
C	100.0	481	481
S	0.0	0	86
T	49.5	481	971

As seen from these limited results, the network tends to choose one value -- vowel, consonant, or silence, as its basis. When viewing the actual activations of the output layer during testing, the networks trained with an alpha of 0.3 and a beta of 0.8 could be seen to attempt to change to the desired output at the onset of a different phoneme type, but

would not quite reach the required level before the phoneme type changed again. This seems to say that a very important attribute of the v/c/s classifier is its ability to quickly recognize that a different type is present and change output accordingly. The duration of each phoneme type is relatively short - sometimes as brief as four records. However, during training the network ouput was seen to closely track the desired output, switching within two or three records to the correct classification. This seems to imply that a better trained and configured network could do the same with a fixed set of weights.

Chossing the run-time parameters associated with the identity-mapping, an alpha of 0.2 and a beta of 0.9, and training the network over 500,000 times, did not produce any recognition improvement. From this several options arise. Among them are the need to increase the number of hidden units and fine-tune the values for alpha and beta.

6. RECCOMMENDATIONS

The identity-mapping network has been shown to function as desired. Further work in this area would include expanding the range of data files able to be trained on and the number of records in the training cycle. This would include modifications to available memory. Also, it would be constructive to audibly compare the processed utterance with the actual utterance to determine if any artifacts have been introduced.

The vowel/consonant/silence classifer needs extensive testing to determine its effectiveness. All that has been gained from this effort is the means by which to conduct this testing and collect effectivenss statistics. As a starting point, a higher number of hidden units should be investigated.

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Contract No:	F49620-88-C-0053

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DATE:	April 16, 1989
Contract No.:	F49620-87-R-0004

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Working Memory and Cognitive Structure

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FINAL REPORT

Evaluation of Air-Intercept Performance: Observer Reliability Issues

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Contract No:	F49620-88-C-0053

Same Report As
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FINAL REPORT

Integral Displays in Interactive Dynamic Environments

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Integral Displays in Interactive Dynamic Environments

by

Mona L. Toms

ABSTRACT

Prior research has indicated integral displays are effective in tasks where information needs to be integrated prior to a response. A literature review of research in this area has found that the effectiveness of integral displays has not been investigated in interactive dynamic environments that characterize command and control, logistics, and air defense domains. This report discusses problems that are characterized by these types of environments, identifies relevant research issues, discusses how integral displays may facilitate as decision support, and recommends an experimental design to test these issues.

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I. INTRODUCTION:

A. Overview.

Despite the increasing trend toward automation, the human operator will most likely still be involved in the control and regulation of dynamic systems. This task often involves the integration of information from multiple sources which is time-varying in its relation to a criterion. Decision makers in such environments are typically uncertain of which decision rule is applicable because the decision rule changes given the current state of the system. Because systems are becoming increasingly complex, it is becoming more difficult to make inferences about future system states and determine appropriate control responses.

The control and regulation of complex systems is made more difficult by the fact that humans are suboptimal in combining multiple sources of information under uncertainty. Research findings (Tversky & Kahneman, 1974) indicate that humans have certain known biases in the decision making process. Studies have shown that humans not only find it difficult to use more than one criterion at a time, they tend only to identify and use criteria that will support a current hypothesis.

There has been increasing emphasis on ways decision making and thus performance can be optimized with the use decision support systems. One way is to assist the operator in developing a mental model for an accurate understanding of how changes in the system components interact and effect system state. Another way is to provide support with the use of decision aids that would allow information to be easily assimilated. Decision support can be provided on-line in the form of realtime displays or advice or off-line in form of training with these displays or advice. The focus of this investigation is with both the off-line and

on-line aspects of decision support.

B. Graphical Representation of Data.

The decrease cost of hardware and the increased flexibility of software has contributed to the prevalent use of graphics in decision support. By representing data in a graphical format, complex data can be organized in a format which allows the user to easily extract meaning from the display. A graphical representation is thought to be effective because it capitalizes on the excellent pattern-matching capabilities of the human; and as a result, the graphical interface is capable of providing "powerful ways of bringing abstract things into the realm of the perceptually knowable. "(Holland et al., p. 136).

The graphical display of multivariate quantitative information can be represented in many forms. Conventional formats include bargraphs, linegraphs, scatterplots, and trend plots. While these displays integrate information to an extent by grouping relevant information, they are considered separate in the sense they use different displays or objects to display multivariate information. Research has shown that information can be further integrated by mapping mulivariate information onto several dimensions of a single object.

One way of representing multivariate information is with the use of geometric objects; such as rectangles, triangles, and polygons. These displays, referred to as object or integral displays, facilitate in the identification of system state with a particular configuration of the object representing a particular state.

C. Integral Display.

The integral or object display has shown considerable promise of facilitating the integration of information from multiple sources; and will be the

focus of this investigation. An example of a object display is the SPDS (Safety Parameter Display System) display that is used in nuclear power plants (Woods, Wise, Hanes, 1981). Eight system system variables are represented as spokes which are equidistant from the center of the figure. The reference points are scaled so that the reference pattern is always an octagon in normal operating condition. Transformations in the octagonal pattern represent abnormal states.

There are several advantages for the use of an integral display in an information integration environment.

1). It acts as a filter of information by restricting the display to only data that needs to be integrated for a response.

2.) Facilitates integration of information that humans are not good at doing, such as nonlinear (Slovic and Liechtenstein, 1971) ; or because of biases (Kahneman & Tversky, 1974) or performance constraints.

3.) Reduces panel space requirements such that a single display may replace several displays; and as a result, visual scanning is also reduced between displays.

4.) Facilitates rapid recognition of total system state.

There are several cognitive reasons postulated for the integral display advantage in integrating information. Recent research (Sanderson et al., 1989) has indicated that a key factor may be its use of what Pomeranz (1981) refers to as an "emergent feature". An emergent feature is defined as a property of a configuration that emerges when multiple dimensions are combined that did not exist when the dimensions were independent of each other.

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II. OBJECTIVES OF THE RESEARCH EFFORT:

A review of research reveals that there has not been any research that has investigated the effects of integral displays in interactive complex environments (to this author's knowledge). My assignment as a participant in the 1989 Summer Faculty Research Program (SFRP) involved working with Dr. Kevin Bennett in the development of a part-task trainer. As a research goal for this summer fellowship, I investigated the use of the integral display as one way information can be integrated for display design to reduce task complexity and enhance learning in the part-task trainer. Several objectives were stated in meeting this goal: review research issues and findings, identify an area of relevant research that needs further study, and design a pilot study in the identified area. These objectives were met, and in addition, a pilot study was implemented of which the results are discussed in this paper.

A literature review of past research has investigated the effectiveness of integral displays in simplistic, non-interactive environments. The findings typically have found an advantage for the integral displays in tasks which require information to be integrated for a response; and a cost for integral displays in tasks which require attention to be focused on a single informational source, or attention to be divided between informational sources for independent responses. Tasks usually require subjects to make a criterion judgment response in which they did not interact with the system. In most realistic environments, such as command and control and process control, the integration of information for an identification of system state and control input are much more complex.

Our research objectives are to investigate the effectiveness of a rectangular integral display in a complex control task. Several issues have been identified as relevant to this research: 1) Would an integral display enhance performance by facilitating integration of information in a complex control task? 2) Embedded in this first issue is the issue of decomposibility . Would a complex rectangular display permit decomposition of individual attributes in this type of task? 3) Would learning be enhanced with an integral display by facilitating an understanding of complex component relationships? 4) Would the integral display show more of a benefit in this type of environment as the task became more difficult?

III. REVIEW OF PAST RESEARCH:

Past research has investigated the effectiveness of integral displays in non-interactive environments with transformations of the component variables experimentally controlled to instigate system failures. Research findings have shown an advantage for integral displays in this type of information integration task. The following summarizes the results and methodologies of these studies.

Goldsmith and Schvaneveldt (1984) with the use of static displays found integral display advantages with both a rectangle and a triangle over the separable bargraph display in an information integration task. The subject's task was to enter a criterion value of the display according to a predetermined decision rule. Their study found that with a rectangular integral display, practice enhanced performance more with the configural cue relation (complex task) than with the additive relation (easy task). These results indicate that the format of the display as well as practice impacts learning and performance. Performance was measured by correlating cue criterion values with

actual values.

Barnett and Wickens (1988) research with an integral rectangular display suggest that an integral display advantage (for an integrated task) can exist without the associated cost of decoding individual variables. The subject's task was to determine the 'value' of mission by integrating probalistic information that was displayed in in separable (bargraph) or integral display formats. To determine memory of individual components the screen went blank during some portions of the task, and the subject had to recall the value of the individual cues. Performance was measured correlating the actual with the optimal score. Their findings suggest that while the emergent feature (area of the rectangle) facilitate the encoding of information in the integration tasks, it does not necessarily hinder the processing of individual attributes.

Andre and Wickens (1988) found that with a more complex rectangular display, the object display advantage over a separate display of information remains intact. However, the object display does hinder the processing of individual attributes if response time is used as a performance measure. Four sources of information, of which three were used for the task, were mapped onto the dimensions of a rectangle. The subject's task was to estimate the safety stall margin. Performance measures were reaction time and error magnitude. The cost for decoding information in the rectangular display was due to a significant increase in reaction time; however, no significant difference in accuracy performance (er-or magnitude) was found between the displays.

Sanderson, Flach, Buttigieg, and Casey research (1989) investigated the issues of how information is encoded in a graphical representation. The subject's

task was to determine which dynamic system had failed. One system was represented by a bargraph display and the other system by a triangular integral display. Results indicated an object display disadvantage in an integrated task if encoding on the bargraph display resulted in an emergent feature.

Barnett and Wickens (1988), Sanderson et al. (1989), and Wickens and Andre (1988) research have shed new light on integral displays. The Barnett et al. (1988) study suggests that contrary to previous findings with object displays, the dimensions of a rectangle may not hinder the decomposibility of individual attributes. Wickens and Andre research (1988) indicates that if response time is crucial for decoding individual attributes, there may be a disadvantage for an integral display. Sanderson et al. research (1989) suggests the importance of mapping information so that an emergent feature will be perceived relative to the task criteria.

IV. PILOT STUDY:

A pilot study was designed to investigate the effectiveness of an integral display in a dynamic control task. With this pilot study, it is hoped that potential problems in display design, procedure or analysis can be identified before formalizing an experimental design for further investigation. A good test to determine the effectiveness of an integral display is to compare task performance of an integral display with a "non-integral" display of the same variables. Because this is a pilot to determine the overall effectiveness of the integral display in a dynamic control task, decomposibility issues will not be addressed.

The manual control of feedwater in the start-up operations of a nuclear power plant is a complex control task which lends itself rather nicely to the

study of the issues of integration and decomposition with an integral display. This task involves integrating and decoding higher order variables in a complex control task. Through a cognitive task analysis with experts, Roth, Woods, and Gallagher (1986) determined there were several factors which contribute to making this task particularly difficult. Among this are the long time lags, complex thermodynamic processes which are counterintuitive, and a narrow performance envelope.

A. Hypothesis.

It is hypothesized that task performance will be significantly better with the integral display because the task involves the integration of the displayed variables for control inputs. It is also predicted that the integral advantage would increase as a result of increased experience.

The independent variables for this study are: display condition (integral and non-integral) and experimental session (5 days). The dependent variable is time time spend on task.

B. Subjects.

Five subjects, two females and three males, were used for the pilot. All subjects had participated in a prior study which used the separate display of the same variables used in this task. The task was identical to the prior study; therefore, the subjects were experienced with the task in the pilot. Because of time constraints and task complexity, it was convenient to use subjects that had a good understanding of the task.

C. Methodology.

Stimuli: The generic part-task trainer, developed on a Sun Microsystem Workstation, will be used as a model to simulate the dynamics of a nuclear power plant steam generator during start-up operations. The part

task trainer was designed to teach feedwater operators how different primary variables interact and affect the behavior of the system; and how to control and counteract these effects. Three types of displays are investigated with the use of the trainer: a baseline display giving only steam generator level (SGL) information, a feedflow/steam meter display which gives steamflow (SF) and feedflow (FF) information in addition to SGL , and a predictor display which gives actual SGL and predicted SGL. The steam/feed flow display and the predictor displays also gave trend information.

The predictor display provides a prediction of SGL based upon a steady state condition when SF matches FF and eliminates any time lags or the thermodynamic effects of shrink and swell. Shrink and swell effects are energy effects that causes the initial effect on SGL to be the opposite of the actual effect on SGL mass. The predictor display is designed to separate out the relative contributions of two independent processes: shrink and swell effects, and water mass. The differences between the predicted SGL and the actual SGL provide direct information on the contribution shrink and swell.

The simulation incorporates the rate of steam flow as a primary factor in influencing steam generator level. Variations in steam flow is produced by dynamically by altering steam flow in two ways: 1) by continuous adjustments, and 2) through disturbances.

The continuous adjustments to steam flow are partially determined as a function of the contribution of three sine waves. These changes are further augmented by one of three ramp types: a rising ramp, a falling ramp, or a null ramp. The net result of the combination of the three sine waves and the ramp are added to the existing steam flow rate in the model.

The result is a steam flow rate that is either oscillating, oscillating with a gradual rise or oscillating with a gradual fall.

The second variation produced in steam flow is a result of random changes, or disturbances, in steam flow. The number and size of these changes varies as a function of the time in the experimental trial, which could last as long as 5 minutes. For the first 30 seconds of an experimental trial, one disturbance is produced randomly during the interval. During the next thirty seconds, the disturbances are randomly added. The direction of change for a disturbance, either positive or negative, is randomly determined. The size of the disturbance is also varied as a function of time within the trial. Disturbances within the first 30 second time interval range between 0 and 1 %; while disturbances during the last 30 second time interval range between 9 and 10 %. Therefore as the experimental trial progresses, both the size and the number of disturbances increases.

The starting position of the steam generator is randomly determined between the set points of 30 and 70 percent of the steam generator level for each trial. By varying the starting position, it is hoped the subjects would not be able to detect ramp change patterns.

Task: The subject's task for each trial is to control the rate of feedwater flow by pressing one key on the keyboard to increase feedflow and another key to decrease feedflow. The task criterion is to adjust the rate of feedflow so that the actual steam generator level remains between the upper and lower trip points for the duration of the trial, which is 300 seconds. A single trial would continue until the actual steam generator level exceeded the trip points or the trial duration of 300 seconds has expired. At the end of

each trial, the subject is given feedback of the percentage of total time the steam generator level was maintained between trip points.

Procedure: A within subjects design was used with the integral and nonintegral conditions randomly determined for each experimental session. The number of trials for each condition was determined by the length of time on the task so that the subject would have approximately the same time with each experimental condition. The experimental session last for approximately one hour, thirty minutes per condition. Testing occurred over a week period with one session per day for each subject.

Integral display: Because of the inherent relationship between SF/FF and actual SGL/ predicted SGL, it is advantageous to use a rectangular display and encode SF and FF on one dimension (width) and encode actual and predicted SGL on the other dimension (height). By encoding SF and FF as two points on the horizontal axis; and actual SGL and predicted SGL as two points on the vertical axis, the distances between these points would reflect the quantitative difference between the two values encoded on each axis. The length of the horizontal lines (width) would represent the quantitative difference between FF and SF. Likewise, the length of the vertical lines (height) would represent the quantitative difference between actual and predicted SGL. The differences between actual and predicted SGL and SF and FF are critical control parameters for maintaining SGL. If differences exist on both dimensions (SGL and rate of flow), a rectangular object would emerge.

The target state for control purposes is have no difference (distance) between FF/SF, no difference (distance) between between actual and predicted SGL, and maintain SGL (actual and predicted) at 50% which

would be represented as a dot. Because of the dynamic nature of the task, this "dot" would be unstable and would emerge as some form of a rectangular object or a horizontal/ vertical line. The overall goal would be to reduce the size of the display. However certain conditions, such as the use of shrink and swell levers, may necessitate temporarily increasing the size of the display.

The complexity of the display also necessitates the use of another dimension of the object to reflect whether SF is greater than or less than FF or whether actual SGL is greater than or less than predicted SGL. Since this is qualitative information, color is an excellent choice for representing this type of information (Cleveland, 1985). The four sides of the rectangle would be a different color representing the four component variables: SF, FF, actual SGL and predicted SGL. Figure 1A represents an example of the integral display.

Baseline Display: A separate display of the four variables used in the integral display (FF, SF, actual SGL, and predicted SGL) is used as a baseline display for the 'nonintegral' condition. To maintain difference information between SF/FF and actual SGL/predicted SGL, SF and FF markers are displayed on one scale and actual and predicted SGL are displayed on another scale. Individual colors for each parameter are the same as in the integral display. Figure 1B represents an example of the separate display.

D. Results.

The results from the pilot study found no significant differences between the two types of displays over the five day period. The overall mean (for five days) for the integral display was 251.4 seconds per trial and for the separate display was 254.0 seconds per trial. There were no overall trends

with either display as subjects became more practiced. The performance increased with the integral display and surpassed separate display performance for the first three days; however the trend reversed for the fourth and fifth days.

E. Discussion.

The hypothesis that task performance will be significantly better with the integral display was not supported in this pilot study. Subjects' comments suggest several possible disadvantages with the the use of the integral display.

1). They were highly practiced with a separate display that used the vertical axis for all displayed parameters. The integral display had the SF/FF information on the horizontal axis.

2). Digital values were displayed for both conditions. At least one subject relied heavily on the digital values ignoring the graphic representations of the separate and integral displays.

3). When there was a difference between actual and predicted SGL and SF/FF were equal, it was not possible to tell whether actual SGL was larger or smaller than predicted SGL. This information is crucial if these values are near trip points.

4). There may have been a stimulus/response incompatibility in the integral display. Feedflow was controlled by an upper key on the keyboard to increase FF and a lower key on the keyboard to decrease FF which vertical movements are compatible with the separate display of information. However SF/FF information moves on the horizontal axis in the integral display. Therefore using a vertical response movement to increase or decrease FF would be incompatible to the horizontal movement of the displayed information.

V. RECOMMENDATIONS:

Although the pilot did not show any differences between the integral and separate display of information, it is possible that the integral display would show an advantage in an active learning environment where subjects have no prior knowledge of the task. Goldsmith and Schvaneveldt (1984) results found that the advantages of an integral display was maximized in this type of environment. By allowing relevant dimensions to be viewed as a unified whole, the integral display could make the relationship between in system components more explicit and thus enhance the learning process.

It is suggested that follow-up research focus on learning issues and use subjects not familiar with the task. Modifications to the study would include: making the response input compatible to the stimulus, modifying the integral display so that actual steam generator level is visible when steamflow and feedflow are equal, removing digital values, and changing the movement of steamflow and feedflow in the separate display to be compatible with the integral display. It is also possible the performance measure used in the pilot was not sensitive enough to reflect any differences between the displays. If a more sensitive performance measure can be determined, it should be used in addition to the time on task measure.

Integral displays may make complex relationships between system variables more explicit in an active learning environment. Research is needed to fully explore the use of integral displays as decision support in an interactive dynamic environments. The part-task trainer provides an enriched environment to test these issues.

Figure 1A. Integral Display.

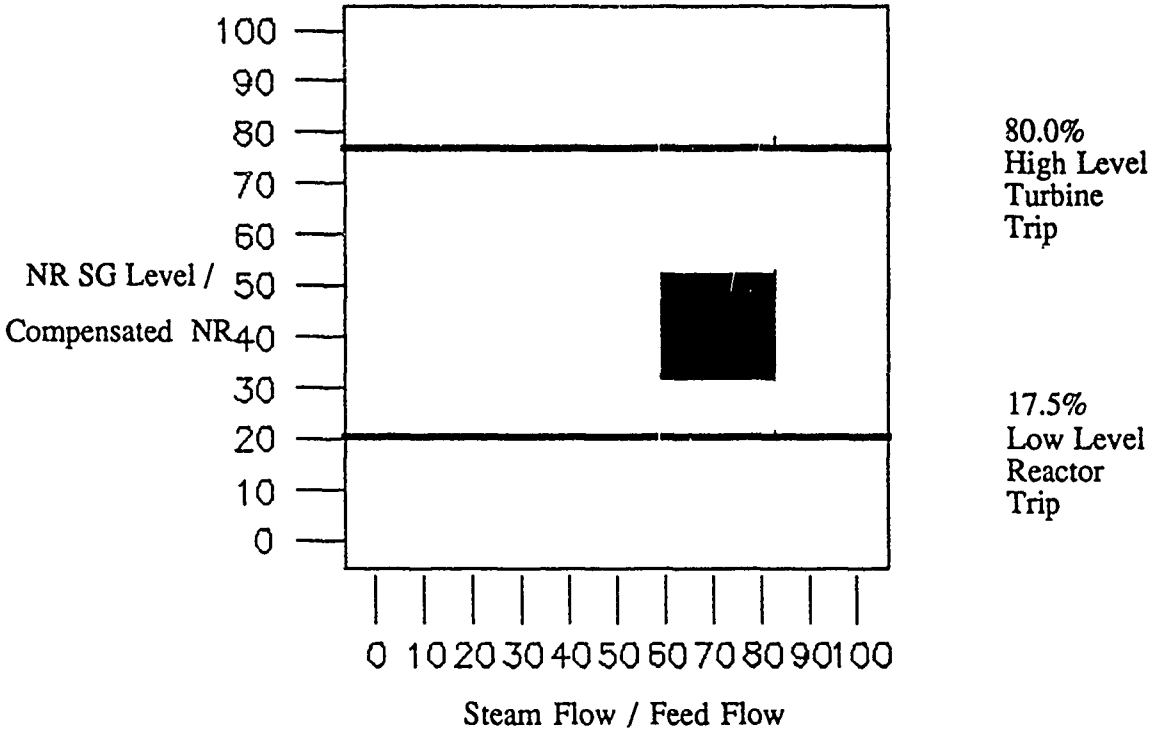
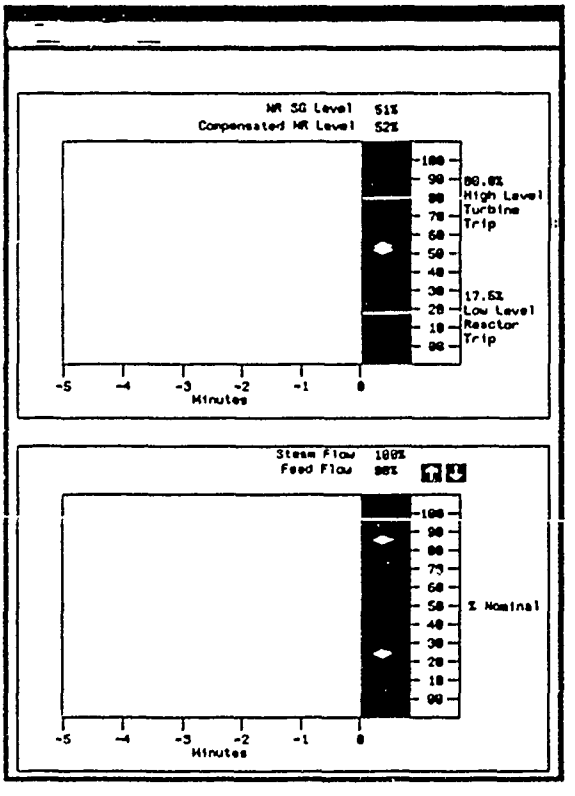


Figure 1B. Separate Display.



1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

GRADUATE STUDENT RESEARCH PROGRAM

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Conducted by the

Universal Energy Systems, Inc.

FINAL REPORT

Software Development to Support Data Collection and Analysis of Cognitive Task Analysis Studies

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Date:	August 10, 1989
Contract No:	F49620-88-C-0053

Same Report As
Prof. Christopher Bell
(Report # 136)

1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

GRADUATE STUDENT RESEARCH PROGRAM

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FINAL REPORT

AN EVALUATION OF STEREOSCOPIC 3D COMPUTER DISPLAYS

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An Evaluation of Stereoscopic 3D Computer Displays

by

John E. Williamson

ABSTRACT

While the principles for creating stereoscopic illustrations have been known for over 150 years, very little applied research has been conducted on determining the benefits of such a presentation. The majority of stereoscopic research has tended to focus on the psycho-physiological aspects of stereoscopic vision in an attempt to localize the cognitive structure which merges the two disparate images. Past applied research has not given conclusive evidence that stereoscopic 3D presented materials improve performance when compared to traditional 2D presentation. Often no difference is found between the 2D and 3D groups, very small samples were used or the results could not be replicated.

Traditionally research into stereoscopic 3D benefits has not examined reaction time as a dependent variable. It is felt that reaction time, rather than accuracy, is a variable which may demonstrate that stereoscopic 3D presented materials can lead to better performance over 2D displays. Several methods of 3D presentation are discussed and a series of experiments which use reaction time are proposed.

Note: The use of the terms "3D" or "three-dimensional" in this paper refers to stereoscopic or true 3D, where the image appears to have volume. Often these images will look as though they are floating behind or in front of the computer screen much like 3-D movies and the popular ViewMaster children's toy. This definition differs from the use of the phrase "3D" in today's computer terminology. This use refers to the representation of 3D objects in a flat 2D space such as a photograph. This technique is often more accurately referred to as "2 1/2D" or solid/shaded (Figure 1).

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I. INTRODUCTION:

The Intelligent Systems Division of the Human Research Laboratory at Brooks Air Force Base examines a variety of technologies which can be applied to intelligent tutoring systems (ITSs). These have included speech recognition, neural networks, expert systems and authoring tools. One of the goals of the branch is to develop ITSs to a point where they provide the most realistic simulation necessary for accurate training. In addition to computer intelligence using neural networks or expert systems, a successful interface must be designed which will allow the most efficient communication between the student and the ITS.

While a great deal of research has been conducted by this branch in the development of intelligence in the ITS, considerably less has been conducted in regard to the interface. A large number of the domains for which ITS will be created in the near future will involve spatial tasks (aircraft maintenance, map reading, etc.). A well-designed interface and the ability to accurately and quickly impart spatial information to a student is very important in such an ITS.

However, the ability to adequately represent a three-dimensional object on a flat, two-dimensional computer screen is rather poor. Several approaches can be taken in attempts to overcome this shortcoming; multiple views of an object can be presented, the object may be shaded, hidden lines removed or the object may be made to rotate upon the student's request.

While all of these methods are effective to a certain degree, each takes a substantial amount of additional computational time or restricts the portion of the screen available for lesson presentation. As a result, the lesson may be slowed down . More importantly, the student may be forced to exert considerable effort in understanding the two-dimensional representation of the three-dimensional object on the screen. This may further impede the progress of the lesson. Valuable time may be spent manipulating the object until the subject understands the image represented. Worse yet, the student may be unable or unwilling to understand the spatial relationship of a particular item and will simply move on to the next topic. This may lead to both decreased performance and frustration. In addition, because the student will deal with actual three-dimensional objects in the field after the lesson is finished, there may be a negative carry-over effect after having been trained with flat, two-dimensional stimuli.

Perhaps if the material can be presented in stereoscopic 3D, the illusion of depth can be used to further aid in understanding the material. This may lead to better retention and comprehension during the lesson itself and better performance on the task in the field.

My research interests have been in those areas of human memory and contextual cues which may lead to improvements in performance in computer/human interaction. I have also begun to examine methods in which the computer can be used to improve performance in the instruction of students in computer aided instruction (CAI) and ITS paradigms. In addition to my computer experience, both in programing and designing interfaces, I have had considerable experience in designing and conducting psychology experiments investigating human performance.

I have also created stereoscopic 3D images using a variety of materials. These have included using a modern SLR 35mm camera, a modern lenticular camera, an antique stereo camera, modified Super 8mm and VHS movie cameras, hand drawn and computer generated images. I have also seen demonstrations of several new autostereoscopic 3D devices and have a working relationship with the inventors. As a result, I have a good understanding both of how 3D images can be created and what constitutes a good 3D image. My unique experience with computer programming, interface design, stereoscopic 3D and experimental design all contributed to my assignment to the Intelligent Systems Division at Brooks Air Force Base.

II. OBJECTIVES OF THE RESEARCH EFFORT:

One of my assignments as a graduate student in the 1989 USAF-UES Summer Faculty Research Program/Graduate Student Research Program was to review the past literature regarding stereoscopic 3D. A review of past literature revealed that little research had been conducted on determining if stereoscopic 3D material is better than traditionally presented 2D material. The research which had been conducted studying this issue did not conclusively support the hypothesis that 3D displays are better than 2D displays.

In addition, I was to evaluate past, existing and emerging technologies for presenting 3D which could be used in an ITS environment. While not all of the alternatives are commercially available, I was able to arrange for demonstrations of prototypes for several new 3D technologies both at Brooks AFB and in Boston at the 1989 ACM SIGGRAPH convention.

Because of the numerous alternatives and additional cost required to display stereoscopic 3D images on a computer screen, an evaluation both of its feasibility and benefits was also requested. I was able to design a study which could begin to test both the feasibility of implementing stereoscopic 3D into ITS and in determining any benefits which may exist for doing so.

A modification of a portion of the experiments suggested in this paper will be conducted beginning this September at Texas A&M University as part of the requirements for the Master's degree in Psychology.

III. REVIEW OF RESEARCH:

Stereoscopic 3D is a method of recreating the perception of depth we see naturally. Because our eyes are separated by 2 1/2", each of our two eyes give us a slightly different view of the world. Through a process still not understood, these two disparate images are merged in the brain and depth is perceived. Stereopsis is a depth cue which we can use to interpret depth information even when other depth cues fail. In addition, stereopsis can be used to make correct determinations of place and form when monocular depth cues (interposition, shading, perspective, etc.) give incorrect or conflicting information (figure 2.)

While the principles for creating stereoscopic illustrations have been known for over 150 years (Brewster, 1856), very little applied research has been conducted on determining the benefits of such a presentation. The majority of 3D research has been conducted to determine the physiological limits of this phenomenon. Past studies have examined such topics as the amount of time which can lapse between the presentation of the two images (Ross and Hogben, 1974,), the minimum binocular angle which depth can be perceived (Ogle, 1953), the delay effect in the Pulfrich phenomenon (Ross and Hogben, 1975), and the prevalence of stereoblindness in the population (Hamori, et al, 1981).

Virtually all of the applied research studying the possible benefits of 3D has been conducted in the last 60 years. Historically, 3D research has gone through three cycles each occurring simultaneously with the rise and fall of the popularity of 3D. The first large volume of research occurred in the 1920's with the last wave of popularity of stereocards. Stereocards are made of two different 3" X 2" photographs placed side-by-side on a heavy piece of cardboard. They are viewed through a hand held viewer based on the design invented by Oliver Wendell Holmes. The viewer consists of a hood to shield the eyes, two lenses both to focus the images and assure that only the left eye sees the left image and vice a versa. The remainder of the viewer consists of a handle and a stage which can be moved toward and away from the viewer's eyes in order to focus the images. Only one person at a time can view the image. During this period, research focused on the use of stereocards in education, as a supplement to books and lectures (Freeman, 1927). As radio and automobile travel became more popular, the 100 year-old stereocard technology quickly died out as an entertainment and education medium.

The second peak in 3D interest was caused by the success of Hollywood's 3D movies during the 1950's. This lead to a huge market for personal 3D cameras and

projectors. Research during this time examined the advantages of 3D movies and slides for education and training (Cogswell, 1952). With the invention of the polarizer, color images could be projected reliably and inexpensively to large audiences using both motion pictures and slides. Each of the left and right eye images were projected through one of two sets of polarizers. Both polarizers were orientated 90 degrees from one another. Members of the audience were required to wear glasses with similar polarizers over each eye. These polarizers were arranged so that the left eye image could not pass through the right eye polarizer, only the right eye image could pass through. With the end of popularity of 3D movies, both 3D research and 3D photography diminished dramatically by the close of the decade.

Currently, we are in the third period of research on 3D. The computer industry's desire for more realistic images has rekindled the interest in 3D for flight simulators, computer aided design (CAD) packages, entertainment, and medical imagery. Several companies currently produce 3D software and display devices costing between $50 and $250,000.

The most successful computer display devices today present 3D images in a manner similar to the polarizer technique employed in the 1950's. The only difference is that only one image is presented on the screen at a time rather than simultaneously. The left and right eye images are flashed alternately on the screen at 60 frames per second. A liquid crystal shutter is place between the computer screen and the viewer. In the Stereographics design, the viewer wears the shutters and the left and right shutters open and close in sync with the views on the screen, assuring that the left eye view is only seen by the left eye. The other variation on this technology, used by Tektronix, is to have the liquid crystal shutter polarize the light from the computer screen rather than block it completely. This allows the viewer to wear passive polarized glasses, similar to sunglasses, rather than the active glasses required by the Stereographix design. This is a more expensive approach initially, but cheaper for multiple viewers.

Other 3D display devices are currently being marketed or developed by several companies. These are not as successful commercially or aesthetically as the liquid crystal shutter devices from Stereographics and Tektronix. Two autostereoscopic devices from Texas Instruments and BBN Systems do have the potential for future implementation. They are called autostereoscopic because the viewer is not required to wear glasses of any type in order to see depth in the image. The viewer may also walk around the display and the see the drawing from any angle. They both function by drawing slices of the image on a rapidly moving plane. In the device from Texas Instruments, the plane is a spinning disc mounted at an angle. In the BBN implementation, the plane is made of a mirror which flexes in and

out similar to the movement of a sound speaker. Neither is currently able to draw solid shapes, only wire-frame line drawings are possible. Other display devices including anaglyphic, lenticular screens and virtual world helmet mounted displays were also investigated. These alternatives had limitations regarding the types of drawings they could produce and the cost. It was felt that the liquid crystal shutter systems were far superior in terms of cost and quality of output.

All three periods of 3D research have given inconsistent results and have often found that 3D presented materials may not aid in performance or understanding. This body of research has to a large degree focused on determining if depth can be perceived in a variety of new techniques. Relatively few studies have examined if this added depth information can be used by observers or operators and lead to better performance.

The applied focus of 3D research has examined a wide range of display devices. Past and present studies have included holograms, fluorescent cubes, spinning arrays of LED's, lenticular screens, Pulfrich phenomenon, lasers illuminating both vibrating mirrors and spinning platters, LCD shutters, polarizers and such traditional means as anaglyph and stereo pairs. It is felt that before additional money is spent on development of these and new 3D display devices that research must be conducted to determine their usefulness in an applied setting.

The lack of findings which show a performance advantage for 3D displays may be explained in part by the experimental design used. None of the past studies have examined reaction time as a dependent variable. Only studies in remote arm manipulation have examined time as a dependent variable. However, these examined the overall time required to complete a variety of tasks in a remote arm manipulation situation (Fujita, et al, 1986, Draper, et al, 1988, Crooks, and Coan, 1977). This is a different measure than looking at reaction time for discrete, individual tasks such as target detection, recognition and object avoidance (Ewrin, 1974). Because the benefits for 3D presentation may only be found in split-second reaction times, this effect may vanish in a long series of tasks.

In addition, only two studies have examined memory benefits for 3D presented materials. Neither found that 3D had any benefits. Again, neither used reaction time as a measure. It is hypothesized that because of the added depth cue, memory (recognition), may be superior for 3D presented objects than for 2D. This superiority may be seen in faster reaction times rather than accuracy measures. This may due to the fact that the use of monocular depth cues (interposition, shading, linear perspective, texture gradients) enable subjects to successfully complete a recognition task with a high degree of accuracy. These monocular depth cues supply sufficient depth information for accurate recognition as is evident by their prevalent use in displays.

It is not surprising that past research has failed to conclusively demonstrate that 3D outperforms 2D. Past research has tended to look only at whether the task can be completed or not using 3D and if any difference in accuracy exists. Naturally, the tasks they have examined can be performed very well in 2D or they would not have been performed that way for so many years. As such, the past research may have obtained a ceiling effect. In addition, past research has often had very small sample sizes (2 or 3 subjects is not uncommon), the experimenters themselves often doubled as subjects, and control groups were inadequate or missing. A body of well designed research is needed that will examine differences which may exist between 2D and 3D materials using variables which have not previously been measured. It is felt that both reaction time and recognition measures will accomplish this.

If reaction time can be shown to be significantly faster in conditions where 3D displays are used, this will provide support for he continuing development of 3D displays in general and specifically in ITSs. In addition to the possibility of learning enhancement, the question of faster reaction time may be of special interest to the military because of the importance of split-second reactions in hostile combat situations. The added time saved in target recognition/avoidance which may exist in 3D displays may be enough to justify the added cost of the stereoscopic display in situations other than training.

IV. RECOMMENDATIONS:

It is recommended that the series of simple, easily constructed experiments outlined below be conducted in order to determine the effectiveness of stereoscopic 3D presentation in an ITS environment. It is felt that either of the two liquid crystal display devices will perform to a level suitable for ITSs and at a price which is within the range acceptable for multiple workstations. Each of the experiments addresses issues which have not been approached in the past. In addition, the experiments are done in such a way that the results are easily and accurately applied to everyday situations. A U.E.S. R.I.P has been submitted with the support of Dr. Wes Regain at AFHRL/IDI to examine these issues.

a. Requirements

The experiment must be designed to be run with a minimum of hardware modifications and additional equipment purchases. In addition, it is preferable if more than one subject may be run simultaneously and that a procedure be used which does not require a great deal of training. Therefore, it was recommended that the liquid crystal shutter system be purchased to investigate both the feasibility and benefits of using a stereoscopic 3D display as an interface in ITSs. The liquid crystal technique gives the greatest flexibility and

best image considering the cost. In addition, a wide variety of images from other software packages can be incorporated.

b. Subjects

The subjects will be taken from the Air Force training program in San Antonio and will be needed for 2 hours to complete the tasks outlined below. Several "extra" subjects are needed because it is anticipated that some subjects will need to be replaced because they may suffer from stereoblindness. Stereoblindness is the inability to see depth from stereo (two-view) images. Estimates of stereoblindness or stereovision impairment in the general population range from 5% (Newhouse and Uttal, 1976) to 30% (Richards, 1970).

c. Materials

Zenith IBM AT compatibles owned by the Air Force Human Resources Lab can be used, both to collect the subjects' responses and to present the material to be examined. Each computer is already equipped with a hard disk, mouse, and an EGA graphics card and monitor. All software will be written using a combination of computer aided design (CAD), paintbrush, and slide show programs for the graphics screens in addition to Assembler and C for the file and timing routines. The computer's internal clock will be used to record reaction times. The mouse will be used to select targets. The EGA (enhanced graphics adapter) card is used because of it higher resolution and better color selection needed for displaying graphics. Either a Stereographics or Tektronix liquid crystal shutter unit and a multi-sync monitor will also need to be purchased.

The liquid crystal systems draws both the left and right eye image on the computer screen one after another at 60 frames per second. The original images can be drawn using a variety of software packages or even scanned in from video cameras. In addition, it is possible to implement a three-dimension cursor which can manipulate objects in all three dimensions for use in later studies. All subjects in all conditions will wear the glasses with no degradation in image quality for the 2D images. This assures that the glasses are not a factor in interpreting the results.

V. PROCEDURE:

a.Pre-test

The subjects will be shown how to wear the glasses and use the mouse. Previous research conducted by the author has shown that there is initial confusion both in how to use the mouse and in interpreting the depth seen in anaglyph computer screens with novice users.

A brief test for stereoblindness will be given. This will give an indication of the prevalence of stereoblindness in the sample and familiarize the subjects with the effect of the

anaglyph 3D. This test will also acquaint the subjects with the use of the mouse and keyboard.

Ten screens will be shown composed of simple geometric shapes. The subjects are asked to point and click the mouse on the shape which appears closest to them in each of 5 screens. In the remaining 5 screens, subjects will be asked to point the mouse to the shape which is furthest from them. Stereopsis is the only cue available to make these determinations. In two of the screens (one each for lowest and highest), no depth will be present to show subjects the difference between flat 2D and stereoscopic 3D. Subjects will be given feedback if they make an incorrect choice in an attempt to teach how depth is represented in the anaglyph technique. The task will be repeated using a 2-item, forced choice test to familiarize subjects with the keyboard. Subjects will be asked questions such as "Is the SQUARE the highest Y/N?" They will respond "yes" or "no" by using the keyboard.

Several random dot stereograms will also be presented. In this technique shapes which cannot be seen using monocular vision, appear to float above the background composed of random noise. The random dot stereogram will be presented and the subject will be asked a multiple choice question such as "Which shape do you see? A. SQUARE B. TRIANGLE C. CIRCLE D.RECTANGLE?" Those subjects who fail the stereoblindness test will be replaced.

After these tests, the subjects should be familiar with the layout and feel of both the keyboard and mouse and be experienced with the format of experiment. In addition, they should be able to successfully discriminate depth in stereoscopic computer screens. All four of the experiments below will be a within subject Latin Square design assuring that all presentations are counterbalanced.

b. Experiment 1

There is currently a great deal of interest in determining how realistic computer generated images must be in order to improve user performance/understanding (Foley, 1988). An earlier study (Foley, 1988) found that subjects were 20% faster in mental rotation time when solid/shaded objects were used rather than hidden line, wire frame drawings. No additional speed improvement was found when additional processing was done to further smooth the images. Mental rotation was used because it provides a simple experimental design which can be easily controlled and precise data which shows us insight into the subjects' mental processes. Because solid/shaded images may take up to 10 times longer to draw than wire frame drawings, this line of research has important implications in determining the most cost effective technique to use in presenting materials.

Experiment 1 is an extension of this research. In addition to hidden line and solid/shaded illustrations, wire frame and stereographic 3D wire frame and shaded illustrations will be used as well. These two additional types of drawings were chosen to give a more complete continuum of types of illustrations.

It is hypothesized that a steady progression of faster reaction times should be found as the images appear more realistic. Stereoscopic 3D images subjectively appear more realistic than 2D images. It is predicted that wire frame illustrations should be the slowest, followed by hidden line, then stereoscopic wire frame, then 2D solid/shaded objects, with stereoscopic 3D solid/shaded objects as the fastest (figure 1). Again, this line of research is important for determining cost effectiveness, as hidden line illustrations can take up to 5 times longer to draw than the same figure done as an anaglyphic wire frame.

Several variations are made to the traditional mental rotation design (Kosslyn, 1973, Klatzky, 1980) to better test the usefulness of stereoscopic 3D displays in specific applications. For this test the shapes will be rotated in all three planes. Traditionally, mental rotation studies have only examined rotation in the Z plane. This plane allows the objects to be rotated as though they were on a piece of paper and were being rotated on a desktop. This is often referred to as the "picture plane".

This study will examine rotations in the X and Y planes as well. It is hypothesized that in addition to overall differences in mental rotation times for the different types of drawings, rotations in the different planes should lead to differences in reaction times between and within the drawing types (Broota, 1976). Specifically, it is felt that the stereoscopic 3D will yield smaller differences in mental rotation times in the X and Y planes than in the Z plane when compared to the other types of drawings.

Mental rotations typically show a linear increase (Klatzky, 1980, Kosslyn, 1983) in reaction time as the image is rotated further from its original orientation. This is expected to be the case in this study as well. However, it may be found that the stereoscopic 3D does not produce as steep an increase due to the added depth cue. Each screen in the mental rotation task will present the two images side-by-side (figure 3). The target image will be on the right. A total of 60 screens will be shown in addition to 4 screens to train the subjects on the mental rotation task. The subjects will respond to the question "Are these two images of the same object?" by selecting either yes or no on the keyboard. Reaction time and accuracy measure will be recorded.

This will represent the first study examining 3D to use reaction time as a dependent measure. This is an important variable and may explain the lack of significance found in most earlier studies. In a combat situation the added cue of depth may give a reaction time

advantage to the pilot using the 3D system. This may be a critical difference in a battle situation.

c. Experiment 2

The second experiment will be a replication of an earlier, unpublished experiment performed by the author. While not directly related to ITSs, the task is an important extension of the applied use of 3D. One of the potentially largest markets for 3D displays is in non-traditional uses of 3D; that is, modifying displays which currently display 2D information so that they display the same information in a 3D manner. Such displays may include Air Traffic Controller (ATC) displays, where altitude is currently represented numerically. A 3D display for ATC would have the planes floating in space as an indication of their altitude. Novel uses could be found as well, using depth as an indication of threat, importance or complexity in a manner analogous to the flashing warning lights and buzzers currently used. For example, as a target increased in threat, it could begin to float above the screen.(Ewrin, 1978).

A simulated air traffic controller situation will be used in experiment 2 to test this novel use of 3D. Texas Instruments has promoted their new autostereoscopic display as a possible tool for air traffic controllers (Williams and Garcia, 1988) yet no empirical studies have been conducted to determine if this is desirable or more effective than the current ATC screens. Experiment 2 lays the base for testing this application of 3D displays.

The task will be for the subject to identify which airplane is currently at the highest (or lowest) altitude. 40 screens will be used, with 4 training screens. On half of the trails, the word "Highest" will appear on the screen with the airplanes, on the other half of the trails the word "Lowest" will appear. These conditions will be randomly distributed. The subjects will move their mouse to the plane and click the mouse button to select the airplane they feel meets that criterion. 20 of the screens will be in stereoscopic 3D, the remaining 20 will be in 2D. Both reaction time and accuracy will be recorded.

In the control condition the only altitude information available will be the altitude printed below the airplane. In the experimental group, both this number and the anaglyph technique will be available for the subject to use as information on altitude. The numerical representation of the altitude is used in both groups because the stereoscopic 3D method alone cannot represent depth to the degree of accuracy obtainable with the numeric representation.

Both highest and lowest will be tested to see if there is a difference in performance within the anaglyph condition. Self report in the earlier unpublished study indicated that the "Lowest" condition was easier. While it is more dramatic for images to float in front of the

screen in the "Highest" condition, it causes more eyestrain. Several manufactures of 3D displays advise against having objects "violate the window" as it is called, for just this reason (Stereographix Manual, 1988).

d. Experiment 3

The third task is a variation of the earlier mental rotation task. Instead of two isometric drawings placed side-by-side, a three-view orthographic drawing will be drawn with an isometric drawing in the upper-right corner (figure 4). Again, the task will to be determine if the two drawings are of the same object. 4 sample screens will be shown to acquaint the subjects with the task. 40 screens will be shown, 10 for each type of isometric drawings (2D wire-frame, 2D solid/shaded, stereoscopic 3D wire-frame, and stereoscopic 3D solid/shaded). In half of each of these 4 conditions the two types of drawings will be of the same object, in the other half they will be of different objects. All isometric drawings will be at the same rotation and will be counter-balanced in their presentation. Again, reaction time and accuracy will be recorded.

This is a more applied version of the mental rotation task. It was chosen because it represents a real-world problem which needs to be addressed. While architects are able to communicate among themselves using 2D drawings (perspective, isometric, orthographic, floor plans), they often are unsuccessful when communicating with the layman client who has hired them. It is a common complaint that a client will approve a floor plan, and be disappointed at the final product because it is not what they had envisioned the end result to be (Terry Larson, 1988). While it may be unreasonable to train clients (or students) to understand the representation of 3D in 2D media, it may be shown that by using stereoscopic 3D materials that both the architect and client (or instructor and student) will be placed on the same level of understanding of the representation of the object without resorting to building expensive models. As these types of 2D drawings are commonly used in CAI, the same problem may exist in ITS for spatial tasks. Additional military applications which may benefit from the results of this test include training for better comprehension of technical manuals, map reading, and design skills.

e. Experiment 4

The last task for the subjects is a recognition test to examine any memory benefits which may exist for 3D materials. It is hypothesized that because 3D images are more life-like, that in addition to mental rotation benefits, memory benefits may exist as well. Only two studies have been found researching memory benefits for 3D presented materials.

(Vergis, 1954, Damron, 1951). Neither study looked at reaction time, and neither found any superiority for 3D for recall.

Subjects will be shown 20 screens, 10 of which will be 2D solid/shaded drawings; the remaining 10 will be stereoscopic 3D solid/shaded drawings. The subjects will be informed to memorize the items as they will be tested on them later. The test will be a forced choice recognition test presented after an unrelated task to clear the items from short term memory. This test is presented last because it is felt that the subjects should be familiar with the 3D anaglyph technique to the point where it is no longer a novel stimuli. If this task had been the first test, it would not be possible to determine if the result was due to the added depth, or that subjects simply paid more attention to the novel 3D stimuli.

The test will be comprised of 40 screens; half of the screens will be identical to those presented at the learning stage, half will be different. The screens will be in the same format as they were presented originally using a forced choice recognition paradigm. If the drawing was presented initially in 2D, it will be tested in 2D. The subjects' task is to identify which they have seen before. While it would be interesting to examine any cross over effects which may exist for presenting materials in 3D and then testing them presented in 2D, this question goes beyond the current line of research. In addition, for this preliminary study, none of the drawings will be rotated in the test condition. They will all be at the initial rotation presented at learning.

VI. Conclusion

Reaction time has not been tested in 60 years of research examining the benefits of 3D presented materials. This may explain the inconsistent findings when 2D and 3D displays have been used in a variety of studies. It is believed that no differences were found in earlier studies because subjects were able to adequately perform the experimental task using the abundant monocular cues available. It may be that these monocular cues are slower to interpret when used alone than when used in conjunction with stereoscopic depth cues. This proposed series of tests is the first to examine reaction time in an attempt to answer this question.

If significant differences can be found in reaction time, then support for the continuing development of 3D displays will have been found. In addition, a new measure to compare the success of different types of 3D displays will have been developed as a result of this study.

This proposed study begins to examine the largest potential market of 3D; non-traditional 3D displays of both 3D and 2D data such as threat, warning, altitude, size, and distance. This is a wide-open field in human factors which has not yet been examined. This

use of 3D will anticipate the human-factors studies to be conducted on the holographic displays and virtual worlds of the future both for direct applications and in ITSs. It may prove to be inefficient not to use depth as a data display analogous to warning lights and buzzers in the 3D displays and holograms of the tomorrow.

We are currently in the third cycle of popularity for 3D in this century. As computers continue to become less expensive this growth will continue. However, basic research must be conducted to determine if these 3D displays are effective. If this is found to be the case, then research will need to be conducted to determine how to use these displays to their potential both for traditional engineering drawings and entertainment and for representation of existing data in new, creative and possibly better ways.

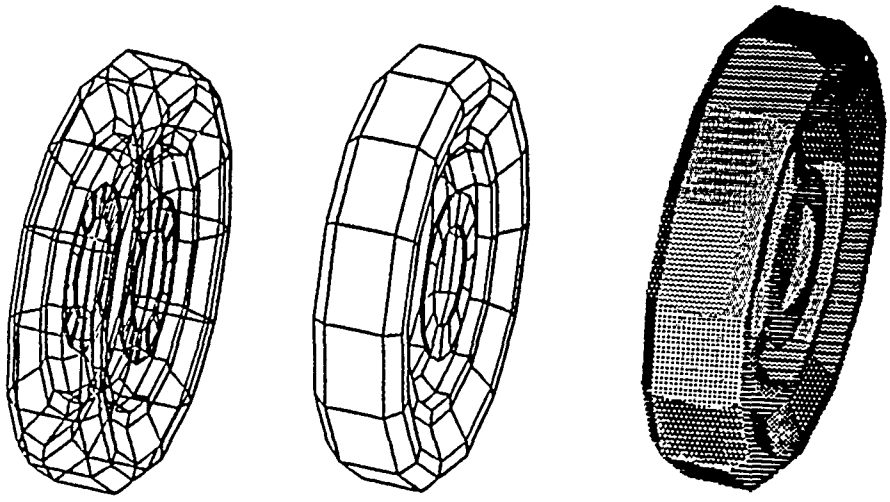

Figure 1.
Wire Frame, Hidden Line and Solid/Shaded Drawings

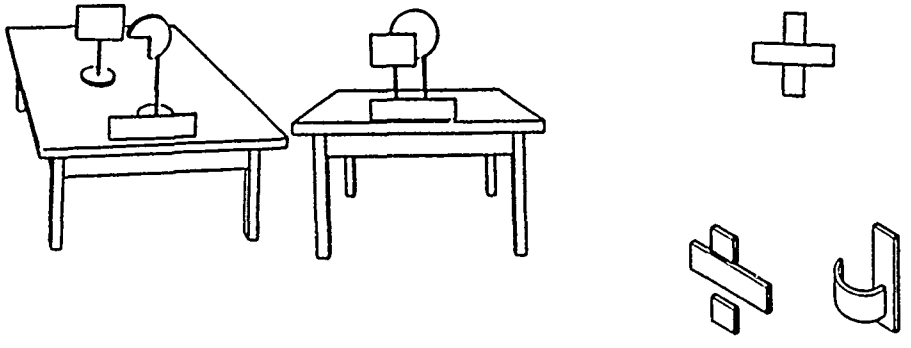

Figure 2.
Possible Interposition Cue Errors

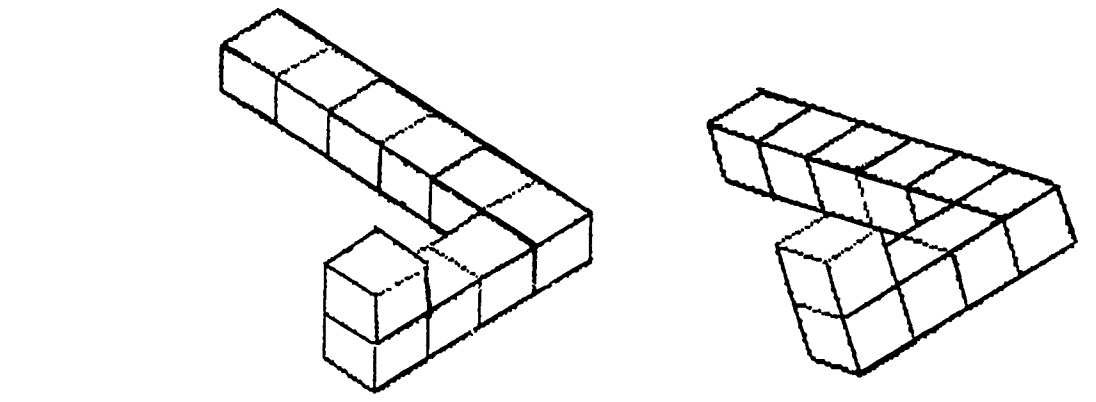

Figure 3.
Mental Rotation Task Example

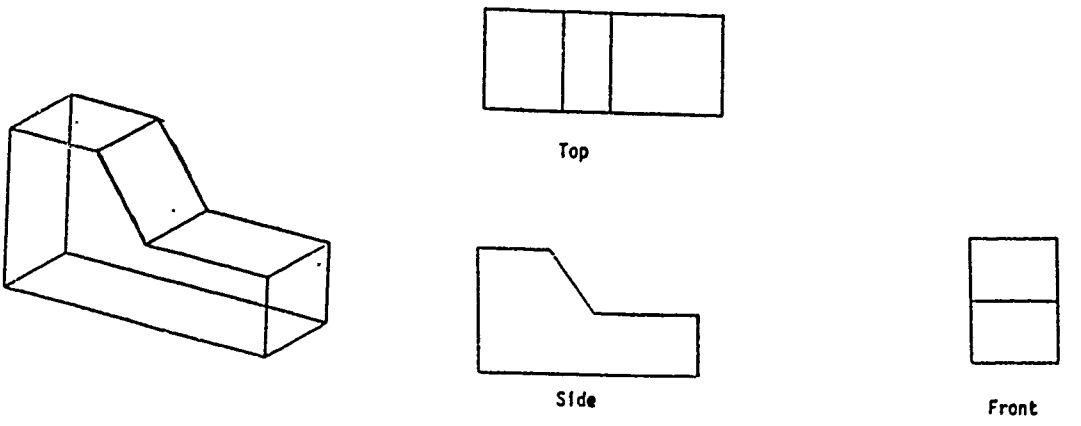

Figure 4.
Isometric and Three-View Orthographic Examples

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FINAL REPORT

BIOLOGICAL ANALYSIS OF THREE PONDS AT PETERSON AFB, COLORADO SPRINGS, CO

Prepared By:	Gregory Zagursky
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University:	Morris College
Prepared By:	William H. Jefferson III
Academic Rank:	Graduate Student
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USAF Researcher:	Lt. Col. Robert Binovi
Date:	20 Sept. 1989
Contract No:	F49620-88-C-0053

Same Report As
Prof. Gregory Zagursky
(Report # 152)

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FINAL REPORT

STATISTICAL ANALYSES OF DATA PERTAINING TO GROUND WATER CONTAMINATION AND LABORATORY QUALITY CONTROL

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Date:	1 September 1989
Contract No.:	F49620-88-C-0053

Same Report As
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(Report # 149)

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Investigation of the Release of Glutamate and Dynorphin A(1-8) by Hippocampal Mossy Fiber Synaptosomes Through Chemical and Electrical Stimulation

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Date: September 20, 1989

Investigation of the Release of Glutamate and Dynorphin A(1-8) by Hippocampal Mossy Fiber Synaptosomes Through Chemical and Electrical Stimulation.

Sudar Alagarsamy

Abstract

The hippocampi of male Hartley Guinea Pigs were dissected, homogenized and subjected to a density gradient separation to yield the mossy fiber terminals. These synaptosomes were exposed to a variety of glutamate agonists and antagonists as well as potassium ion stimulation and electrical stimulation. Four minute fractions were collected from the treated synaptosome preparations and the superfusates were analyzed for glutamate and dynorphin A(1-8). Quantification was accomplished by an enzyme mediated fluorescence assay to determine the amount of glutamate and a radioactive binding assay to determine the amount of dynorphin A(1-8).

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The help of my effort focal point, Dr. Russell Burton was also indispensable as was the assistance of the laboratory technicians, Peter Hernandez and Anna Marie Michel and the high school apprentice Chrissy Cheney.

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I. Introduction

My research interests include the biochemical foundation of emotion and behavior. The neuroscience studies being conducted at the Air Force School of Aerospace Medicine headquartered at Brooks Air Force Base in San Antonio have afforded me the opportunity to investigate some interesting issues relevant to my proposed field of study. My interest and involvement in the computer simulation of neural connections, my studies in biochemistry, biology, psychology, as well as my previous work at Brooks Air Force Base have contributed to my research efforts in the Neurosciences Function of the Clinical Sciences Division. The studies being done at Brooks include the use of the mammalian hippocampus as a model for the physiological basis underlying the process of learning and memory.

The hippocampus is thought to be essential for memory, and therefore, the hippocampus must play an integral part in learning. (1) There is one hippocampus per hemisphere of the brain. The hippocampi are dissected free and subjected to treatment which results in the isolation of the mossy fiber synapses. (2) The animal chosen for the experiments described here is the male Hartley guinea pig. The isolated synapses are referred to as synaptosomes. The synaptosomes are exposed to a number of experimental perimeters and the neurotransmitters which are subsequently released are quantified. The neurotransmitters quantified in these studies are glutamate and dynorphin A(1-8). Glutamate is an endogenous dicarboxylic

amino acid. The researchers of this laboratory have uncovered evidence that suggests glutamate may be the primary amino acid neurotransmitter at the mossy fiber-CA3 synapse. (2) Dynorphin A(1-8) is a neuropeptide opioid which is co-released with glutamate from the mossy fiber terminals.

The outcome of the experiments being performed at Brooks would be essential in understanding the role the synapse plays in long term potentiation (LTP) and consequently in learning and memory. LTP is the extended depolarization of the neurons. This extended depolarization may cause a conformational change in the synapses. This conformational change is thought to occur in the hippocampus. The change may result in the receptor sites of the changed synapses to respond to different levels of the activating neurotransmitter or they may even by activated by totally different neurotransmitters after the conformational change. This change may be the physiological basis of learning and memory.

II. Objectives

One of the objectives of this ten week appointment was to investigate the possibility that the release of glutamate and dynorphin A(1-8) may be distinguished on the basis of the frequency of the stimulus delivered to the synaptosomes. Glutamate should be preferentially released at relatively low impulse activity. This hypothesis is based on the observation that the pattern of presynaptic activity can determine the type of transmitter released and that the threshold for the release of neuropeptides appears to be considerably higher than for other transmitters co-released from the same nerve terminals. (3) Thus dynorphin, a neuropeptide, should be released by a high threshold mechanism in response to electrical stimulation. The distinction of glutamate and dynorphin on the basis of frequency dependence is derived from the finding that there are large, dense-cored vesicles containing neuropeptides in the synapses. (4) These vesicles may fuse to, and release peptides from, morphologically nonspecialized sites distant from active zones in response to electrical stimulation. Therefore, if the voltage-dependent calcium channels are highly localized at the active zones of the presynaptic membrane, the depolarization of the active zone would cause the calcium ion influx necessary for the dense-cored vesicles to fuse to the presynaptic membrane would have to be more pronounced than for those vesicles in the inactive zone.

Another objective of the appointment was to investigate presynaptic receptors for excitatory amino acids in the guinea pig hippocampus. This group of amino acid receptors are named for the selective agonists and include N-methyl-D-aspartic acid (NMDA), kainic acid and quisqualic acid. (5) Glutamate activates all three receptors while each of the other three agonists are relatively selective for their respective sites. If the mossy fiber terminal has a presynaptic glutamate autoreceptor then, agonists may activate the receptor site and inhibit or induce the release of transmitter from the mossy fiber synaptosome. Glutamate antagonists should prevent the agonist activation of the receptor site. Therefore, analyzing the amount of glutamate released in the presence of glutamate agonists and antagonists will help confirm the existence of inhibitory or excitatory presynaptic glutamate receptor sites. Since dynorphin is co-released from the presynaptic terminals, evaluating the dynorphin content of the superfusates collected from the treated synaptosomes will help in evaluating the nature of the receptors.

III. Methods

The methods used to test the electrical stimulation hypotheses involves stimulation of mossy fiber synaptosome release of elevated levels of glutamate and dynorphin A(1-8) by both, extracellular potassium chloride and electricity. The superfusates from the stimulated synaptomes will be collected at four minute intervals. In addition, a few fractions will be collected prior to stimulation to provide a baseline reading for both glutamate and dynorphin A(1-8). A few fractions will also be collected between stimulation by potassium chloride and electricity. The electrical stimulation involves the use of pulses of constant voltage and varying frequency and duration. The superfusates will be analyzed for their glutamate and dynorphin content.

The methods used to test the autoreceptor study involved the use of several plastic syringe barrels which served as perfusion chambers. The syringe barrels were packed with the mossy fiber tissue preparation over a layer of sephadex which was used to hold the tissue in the chamber. The chambers were then subjected to the treatments with agonist, antagonist or combination of both agonist and antagonist. Occasionally, the chambers were pretreated with D-aspartate to exhaust the cytosolic pool of glutamate so the vesicular pool of glutamate could be more carefully investigated. Again, as in the electrical stimulation study, the perfusion solution high in potassium ion concentration is used as stimulated control. In addition high levels of potassium ion is used to test the

extent of the antagonist's effect. If fractions collected from chamber treated with both high potassium ion concentration and antagonist still yield baseline or very low glutamate and dynorphin concentrations then the results of the experiment indicating the antagonistic effect are conclusive.

The procedure used to determine the amount of glutamate and dynorphin A(1-8) involved the use of fluorescence and radioactivity. The glutamate assay was done by obtaining a portion of the superfusate and adding glycine hydrazine buffer. Then at one minute intervals a solution of Nicotine Amide Dinucleotide(NAD) was added to each successive tube and a fluorescence reading taken. After the zero time reading was taken a commercially available preparation of glutamate dehyrogenase (GDH) was added to the medium. Thirty minutes after the addition of the GDH another fluorescence reading was taken on the sample. A standard curve is also performed along with the samples by using known amounts of glutamate instead of the superfusate solution. The emission at time zero subtracted from the emission at thirty minutes compared to the emission values of the standard curve, the amount of glutamate in each of the superfusate samples can be determined. This type of assay procedure was substantiated by performing HPLC on the same tubes on which the fluorescence assay was performed. Both procedures produced similar results, however the assay described here is far less time consuming and sensitive enough to detect the amount of glutamate in the samples produced. This assay is therefore preferable over the HPLC. (7)

The method for determination of dynorphin A(1-8) was more involved. This assay used a competitive binding procedure. The samples were treated with rabbit and goat IgG as well as radioactively (^{125}I) labelled dynorphin over a period of three days. Again, as with the glutamate assay, known amounts of dynorphin A(1-8) are treated like the samples to obtain a standard curve. The radioactivity of the samples compared to the radioactivity of the standard curve indicates the amount of dynorphin A(1-8) in the sample.

IV. Results

The expected results are that the electrically stimulated fractions will have a higher glutamate concentration than those fractions which were not stimulated. Dynorphin concentration in the superfusate should increase with increasing frequency of stimulation. Potassium ion stimulation is used along with electrical stimulation since it is known that high potassium ion concentration in the perfusion buffer cause release of both glutamate and dynorphin by the mossy fibers. (1) And thus, the fractions which were stimulated by potassium ion serve as a stimulated control to which the electrical stimulation can be compared. Another reason for using both electrical and chemical stimulation is that the viability of the tissue can be assessed. If the high potassium ion solution is delivered after the series of electrical shocks and the potassium still causes release of glutamate and dynorphin it can be confirmed that the tissue survived the trauma of the electrical stimulation.

The results obtained from the electrical stimulation project did not yield satisfactory results. The first time the experiment was conducted the results were as hypothesized. (Fig. 1) The superfusate from the electrically stimulated fraction was higher in glutamate concentration than glutamate values calculated for the fractions obtained prior to stimulation. Subsequent experimentation yielded poor results. The glutamate concentration for the potassium ion stimulated

fractions were satisfactory, but the values for electrical stimulation were not higher than the baseline or unstimulated values.

There are many possible causes for the electrical stimulation failing to cause glutamate release, however the most probable cause lies with the design of the chamber used to contain and stimulate the tissue. The thin electrodes used may not have been capable of carrying the charge required to stimulate the tissue. Researchers that have conducted these types of experiments before have used charges of great magnitude. Some laboratories used up to 20 milliamps of current. (6) However the chamber used in these experiments was designed only to carry small currents, a maximum of 5 milliamps. This was because the smaller currents are more physiologically relevant. That is, the lower currents better mimic the action potential and conduction of charges in the neurons in vivo. This is the primary reason for choosing electrical stimulation over potassium ion stimulation. Perhaps the insulating effects of the perfusion buffer used in the stimulating chamber needed to be compensated for, by using a greater charge. Several experiments were conducted with chamber without obtaining release of glutamate or dynorphin by electrical stimulation.

The autoreceptor study was more successful than the electrical stimulation study. The phase of the project focused on during my appointment at the laboratory involved inquiry into the kainate and quisqualate receptors. In general, kainate, caused a marked increase in glutamate and dynorphin

release and quisqualate had the opposite effect. In fact, the lower concentrations of quisqualate actually showed some inhibition of the release of glutamate. (Fig. 2) This effect is an interesting one since it has not been observed by any other laboratory. The increase in the release of glutamate in response to kainate was blocked by various concentrations of CNQX. (Fig. 3) This type of response may indicate a presynaptic autoreceptor.

V. Recommendations

Although the results of the autoreceptor study are fairly conclusive, the true mechanism will not be revealed without further examination of the system. In addition, studies must be conducted to uncover the biochemical link between the autoreceptor mechanism and learning and memory. This link may lie in long term potentiation.

The electrical stimulation project, although inconclusive at this time is also worthwhile pursuing. This type of experimentation is important because of the physiological relevence of electrical stimulation over chemical stimulation of nervous tissue. Perhaps the experiments should be attempted with a larger stimulation chamber able to carry more current. If this proves to be successful, then steps can be taken to lower the levels of current to mimic the amounts of charges carried in living tissues.

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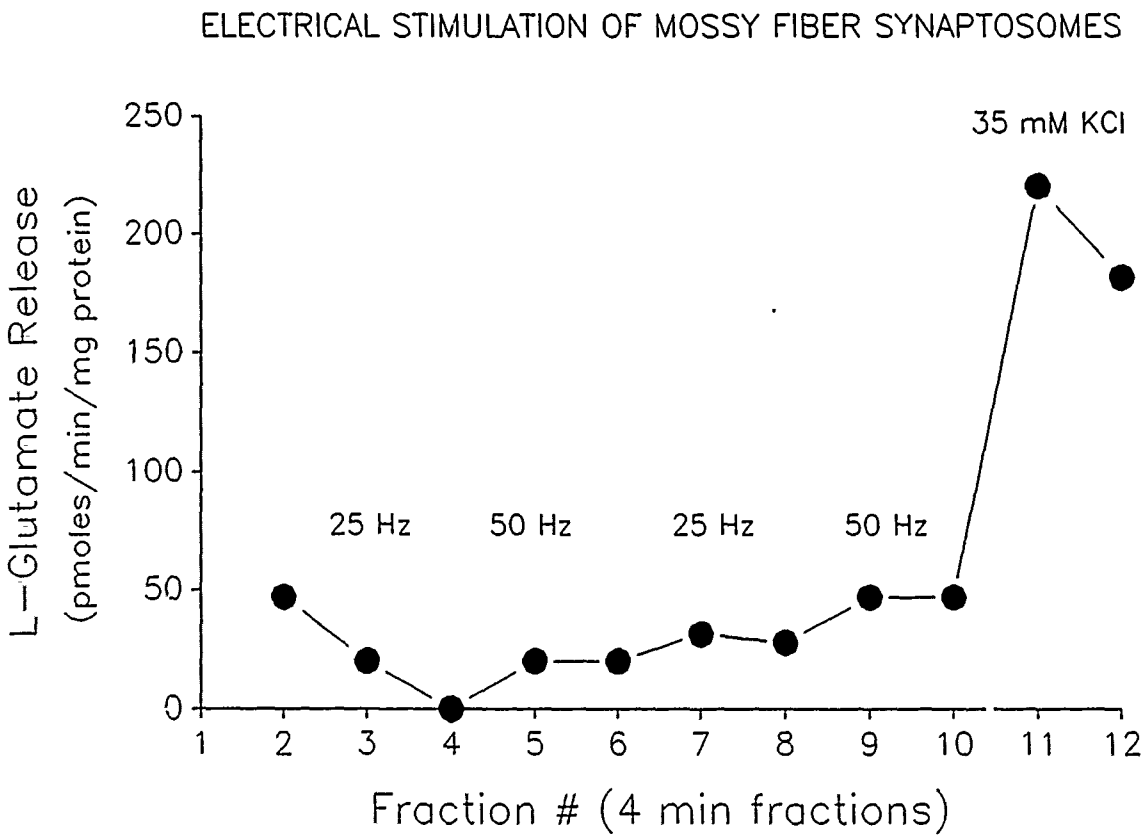
ELECTRICAL STIMULATION OF MOSSY FIBER SYNAPTOSOMES
L-Glutamate Release
(pmoles/min/mg protein)
250
200
150
100
50
0
35 mM KCl
25 Hz
50 Hz
25 Hz
50 Hz
1
2
3
4
5
6
7
8
9
10
11
12
Fraction # (4 min fractions)

FIGURE 1

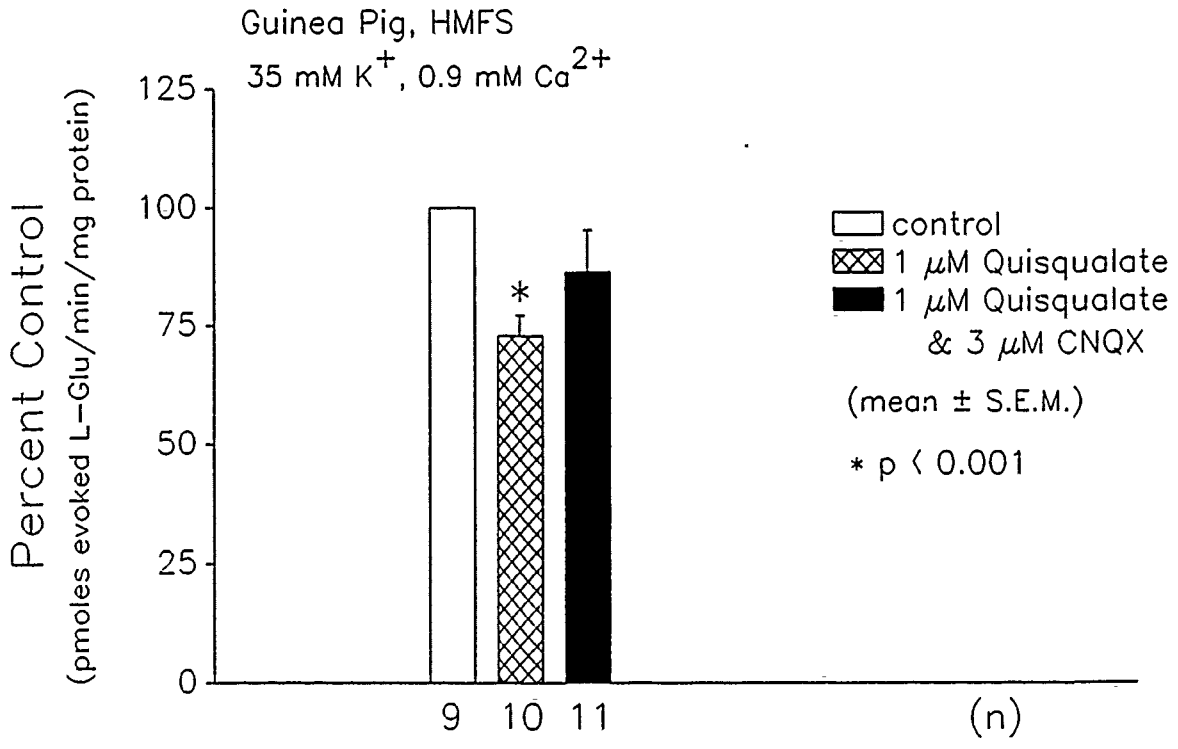
Guinea Pig, HMFS
35 mM K+, 0.9 mM Ca2+
Percent Control
(pmoles evoked L-Glu/min/mg protein)
125
100
75
50
25
0
*
9
10
11
(n)
control
1 μM Quisqualate
1 μM Quisqualate
& 3 μM CNQX
(mean ± S.E.M.)
* p < 0.001

FIGURE 2

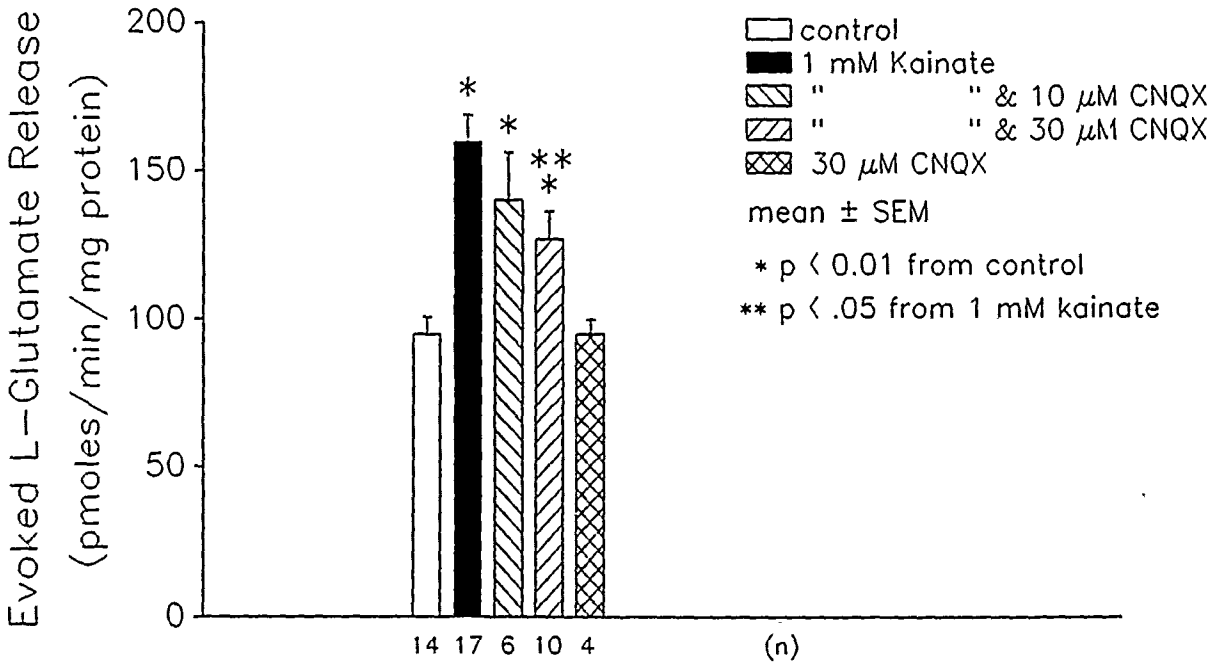
Evoked L-Glutamate Release
(pmoles/min/mg protein)
200
150
100
50
0
14 17 6 10 4
(n)
control
1 mM Kainate
" " & 10 μM CNQX
" " & 30 μM CNQX
30 μM CNQX
mean ± SEM
* p < 0.01 from control
** p < .05 from 1 mM kainate

FIGURE 3

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Final Report

INVESTIGATION OF PICOSECOND PULSES FROM A CW Q-SWITCHED ACTIVE MODE-LOCKED LASER

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Date:	September 30, 1988
Contract No:	F496020-87-R004

INVESTIGATION OF PICOSECOND PULSES FROM A CW Q-SWITCHED ACTIVE MODE-LOCKED LASER

by

John W.J. Barnaby

ABSTRACT

The CW Nd:YAG laser using Q-switching and active mode-locked excitation generates ultrashort laser light pulses(1). These picosecond pulsewidth pulses will be investigated using a Hamamatsu M1952 High Speed Streak Unit and C 1587 Temporaldisperser. In addition photometric data and streak images were graphed for both a 1064 nm beam and a 532 nm second harmonic generated beam.

ACKNOWLEDGEMENTS

Support for this work provided by the Air Force Systems Command and the Air Force office of Scientific Research is greatly appreciated. Administration of the Summer Faculty Research Program by Universal Energy Systems is also acknowledge with apprecation.

The technical and intellectual support given to this effort by John Taboada, PhD, and his able staff have added significantly to the work reported here.

I. INTRODUCTION

Investigation into ultrashort pulsewidth laser phenomenon is an exciting field, expanding at a rapid pace(5). The Lasermetrics series 9500 High Power CW Nd:YAG Q-switched and active mode-locked laser is an excellent source for these picosecond pulses(2,10). Both the 1064 nm and 532 nm laser beams are used extensively in the Vision Research Laboratory at Brooks AFB. My preliminary graduate research dealt with the CW Q-switched active mode-locked Nd:YAG laser. Consequently, working in the Vision Research Laboratory was an excellent opportunity to gain firsthand experience with lasers. Furthermore, the Hamamatsu streak camera gave one the ability to display these pulses for research and study(4,7).

II. OBJECTIVES OF THE RESEARCH EFFORT

The investigation into ultrashort laser pulses of picosecond pulsewidths is a relatively new field(5). There are many different techniques that have been developed and deployed with much success(2,5,7,8,11). The method that was employed used a synchroscan streak camera. The Hamamatsu M1952 High Speed Streak Unit and C1587 Temporaldisperser was used to display the laser pulses on a monitor. The intensity of the laser light versus time had to then be line averaged and thresholded in order to get a "noise free" graph(4). In addition, a procedure had to be developed to transmit the laser light to the streak camera. One of the resultant undesireable traits inherent in such a set up was trigger jitter in the picosecond pulse trains(4,5,7,8). Furthermore, the trigger input on the streak camera required a positive (5-40 Vpp/50 ohm) pulse. Consequently, a positive CW Nd:YAG Q-switched active mode-locked laser pulse detector circuit had to be built.

III. EXPERIMENTAL, RESULTS AND DISCUSSION

The setup for the Hamamatsu M1952 High Speed Streak Unit and C1587 Temporaldisperser was accomplished in a relatively short time period(4). The main problem arose in interfacing the streak camera to the YAG laser. After consulting several references relevant to the experimental arrangement, a preliminary course of action was adopted for transmitting the laser light to the streak camera(3,6,9,16).

The first experimental arrangement for transmitting the laser light consisted of fiber optic cables. This optical fiber transmission system consisted of two cables that were cut and polished. Both ends were polished with the following materials: First silicon carbide 360A, Second silicon carbide 600A, Third abrasive M303, and Fourth Cerium oxide. The first cable was used to transmit the laser radiation to the streak camera. And the second cable was used to transmit the laser radiation to the positive pulse detector circuit mounted on the oscilloscope(8). This detector circuit was then used as a trigger input on the streak camera(4,8). In practice, the problem of supporting the fiber optic cable was a limiting factor in this arrangement. Several different arrangements were tried, but all of them were prone to "microvibrations" inherent in such a set up and its environment.

The second experimental arrangement employed backscattering of the laser light from the first fiber optic cable to the second fiber optic cable. In this setup the first cable transmitted the light from the YAG laser to the EG&G Electro-Optics FOD-100 Ultra-Fast Fiber Optics Reciever. The backscattered light was then coupled into the second fiber optic cable. The second fiber optic cable transmitted the laser light to the input lens system on the streak camera while the trigger signal was transmitted to the trigger input on the streak camera. This arrangement suffered from the same type of problems as the first setup but to a greater degree. The main problem was in coupleing the backscattered light while minimizing the loss due to back-reflection of the laser signal into the external environment.

The third experimental arrangement consisted of using two sets of mirrors to transmit the light to the input lens system of the streak camera. A beam splitter was made out of a microscope slide and was used to reflect a fraction of the laser light to the detector circuit. The mirrors and beam splitter used in this setup were rigidly mounted onto the optical bench and were extremely stable.

The problem of trigger jitter was addressed in several different ways using the basic outline of the third experimental arrangement(4,8). The variable optical delay

was used to control the trigger jitter and consisted of two mirrors and a pellin broca prism mounted on an optical track. The first mirror was used to reflect the laser light to one side of the prism. On the return trip the laser light would exit from the other side of the prism and strike the second mirror. The second mirror would then reflect the laser light to the input of the streak camera. The amount of time delay was controllable by adjusting the distance between the first mirror and the prism and the distance between the prism and the second mirror. See figures and pictures 1-4.

Although the optical delay line was functional, a fair amount of trigger jitter was still apparent(4,7,11). The final solution to the trigger jitter problem was operating the streak camera in single trigger mode instead of continuous mode(2). This enabled the streak camera to get a "snapshot" of the individual pulses in the pulse train. To further improve accuracy in time measurements the setup was rearranged until both edges of the streak tube image coincided with the edges of the video monitor. This allowed the entire slit image to be viewed at one time thus allowing accurate measurements of the pulsewidths for different streak times.

IV. RECOMMENDATIONS

The investigation of picosecond pulses from a CW Q-switched active mode-locked laser offers much more research and study(13,19). There is so much more that one could do with the equipment available in the vision research laboratory(12,14,15,17,18). Because of time contraints, the laser pulses from the Spectra Physics 375B CW Q-switched active mode-locked Nd:YAG laser were barely investigated. See figures and pictures 5-6. One aspect that would be extremely useful to investigate would be the time development of active mode-locking(1,2,10,19). An interesting setup one could use is transient synchronously pumped Q-switched active mode-locked pumped dye laser operation(1). Furthermore, one could study the effects of laser operation with and without Q-switching and with and without mode-locking(1,2,10). Additional setups could be devised depending on the researchers interest and imagination. See figure and picture 7.

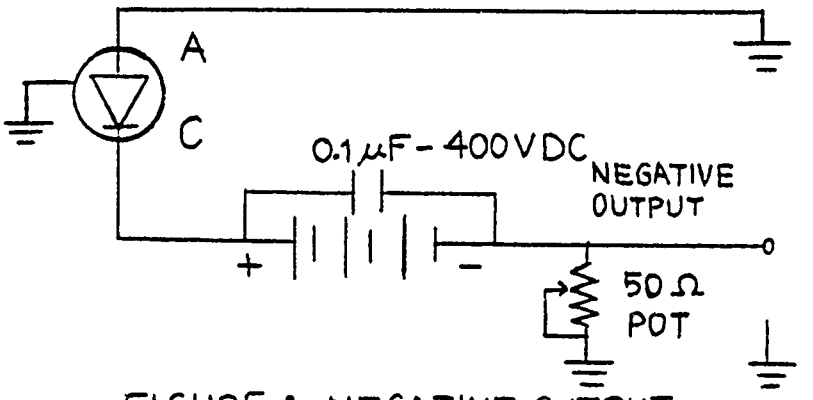


FIGURE : NEGATIVE OUTPUT
PULSE DETECTOR

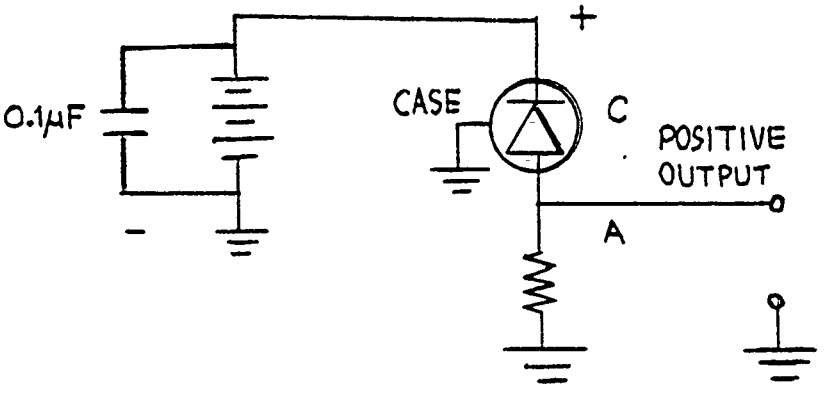


FIGURE: POSITIVE OUTPUT
PULSE DETECTOR

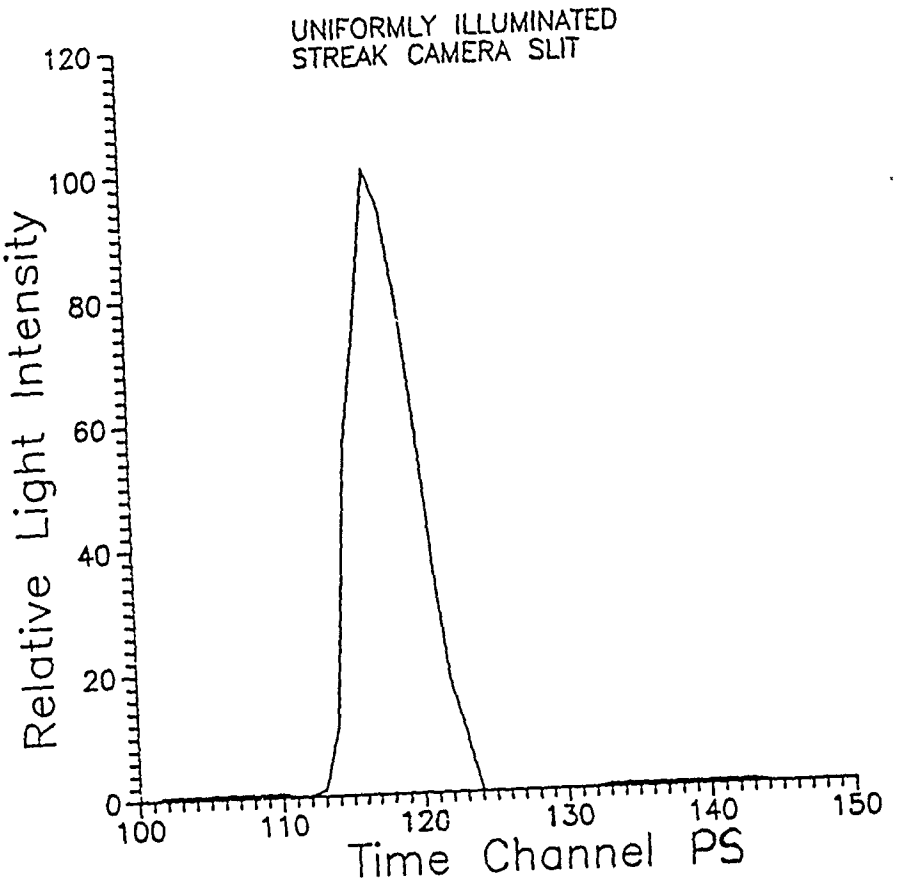
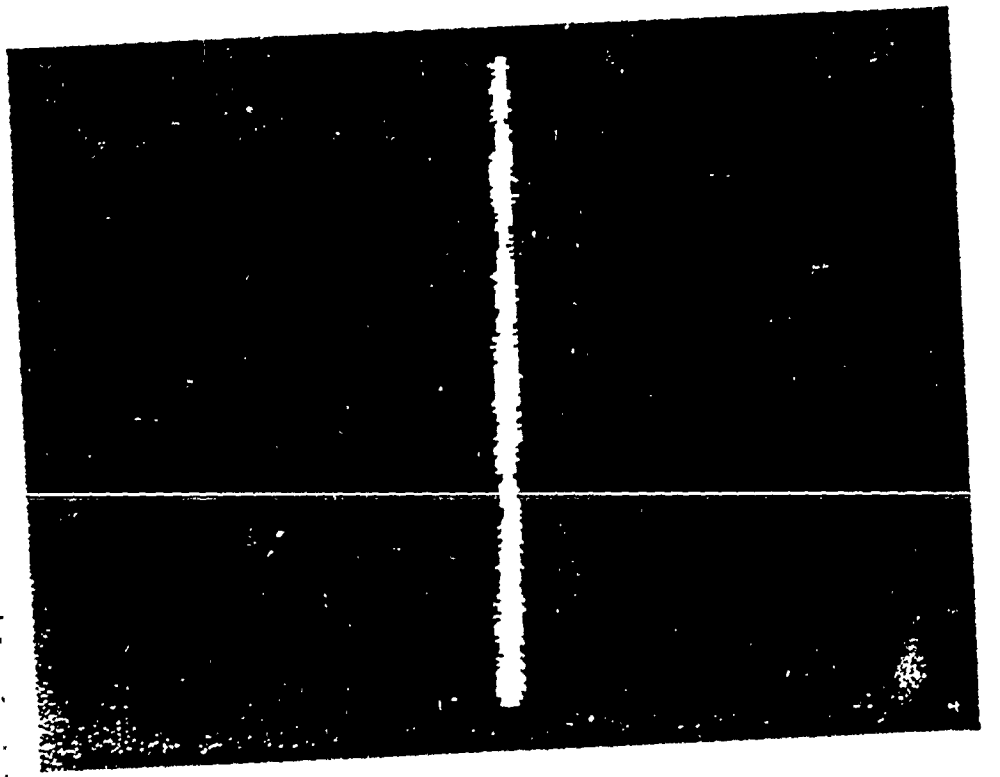


FIGURE 1.



PICTURE 1.

94-11

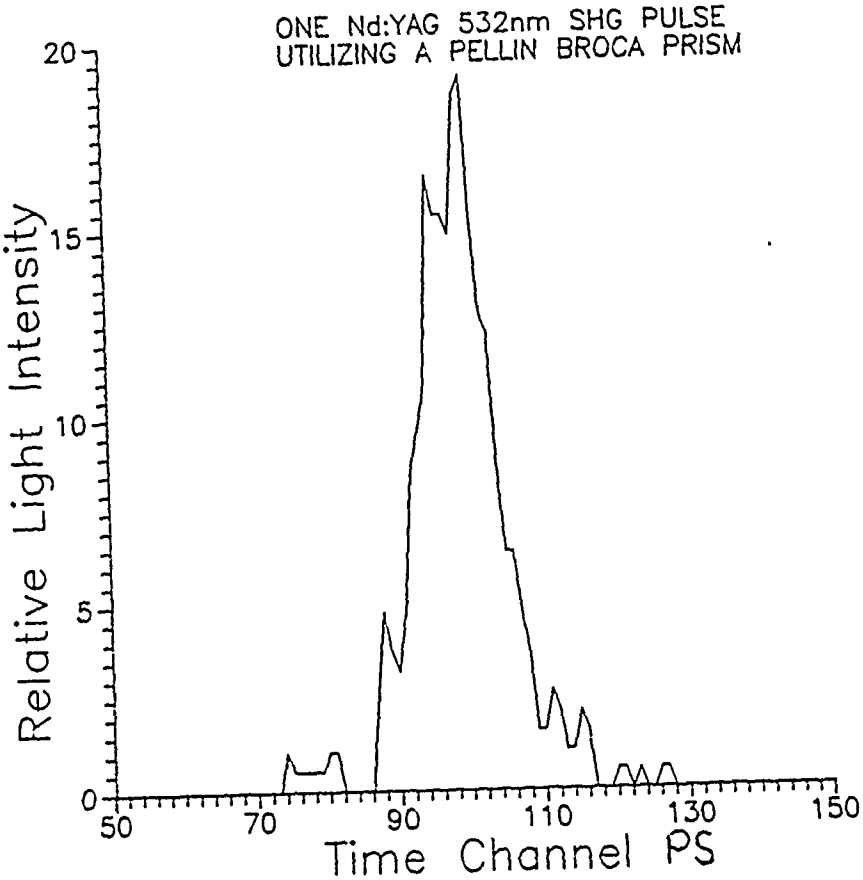
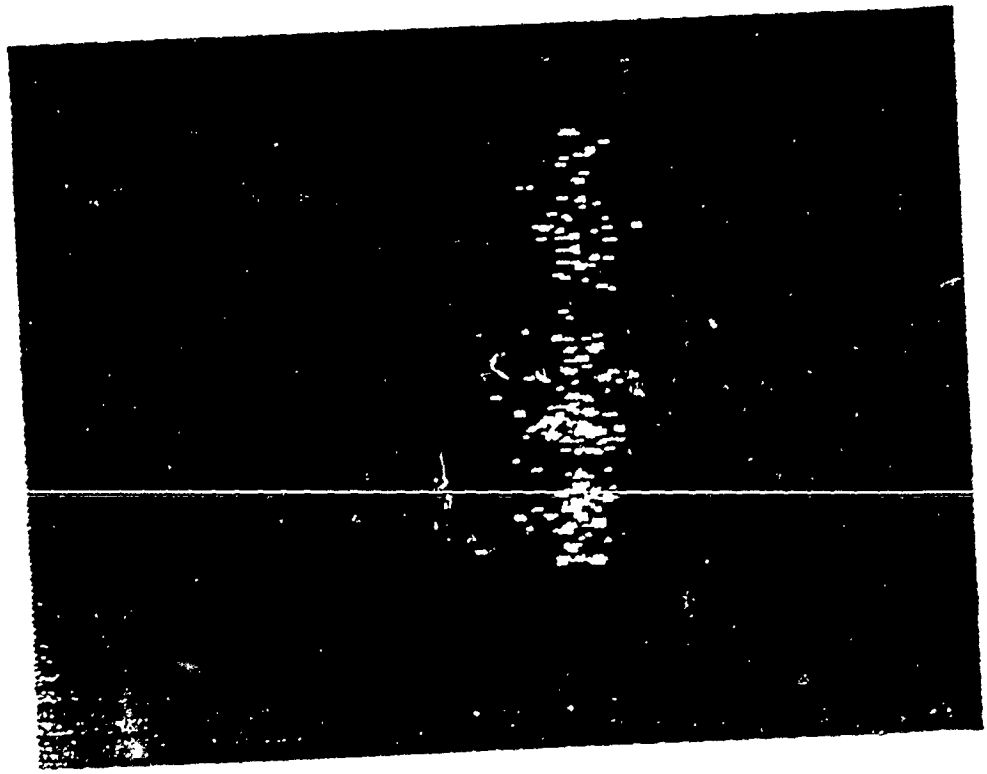


FIGURE 2.



PICTURE 2.

94-12

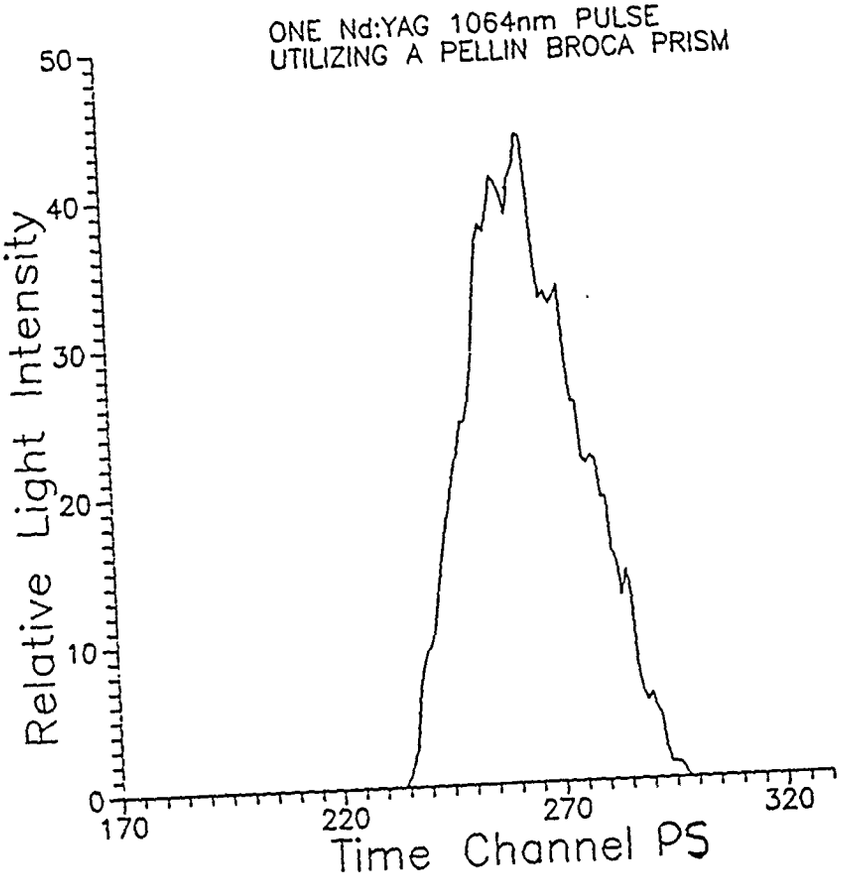
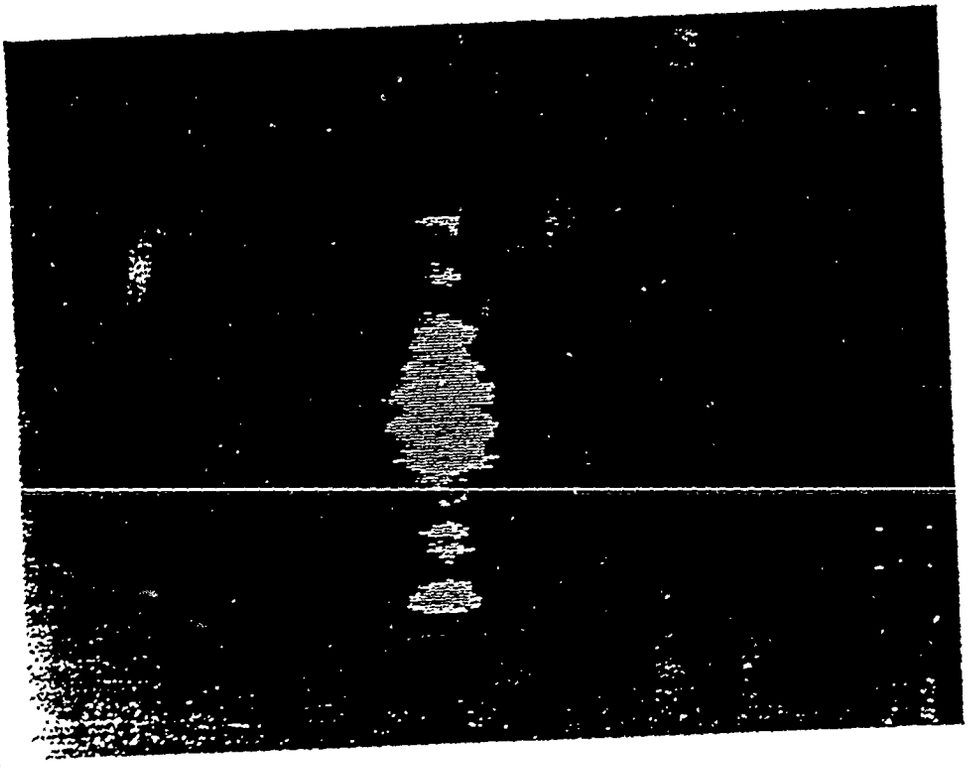


FIGURE 3.



PICTURE 3.

94-13

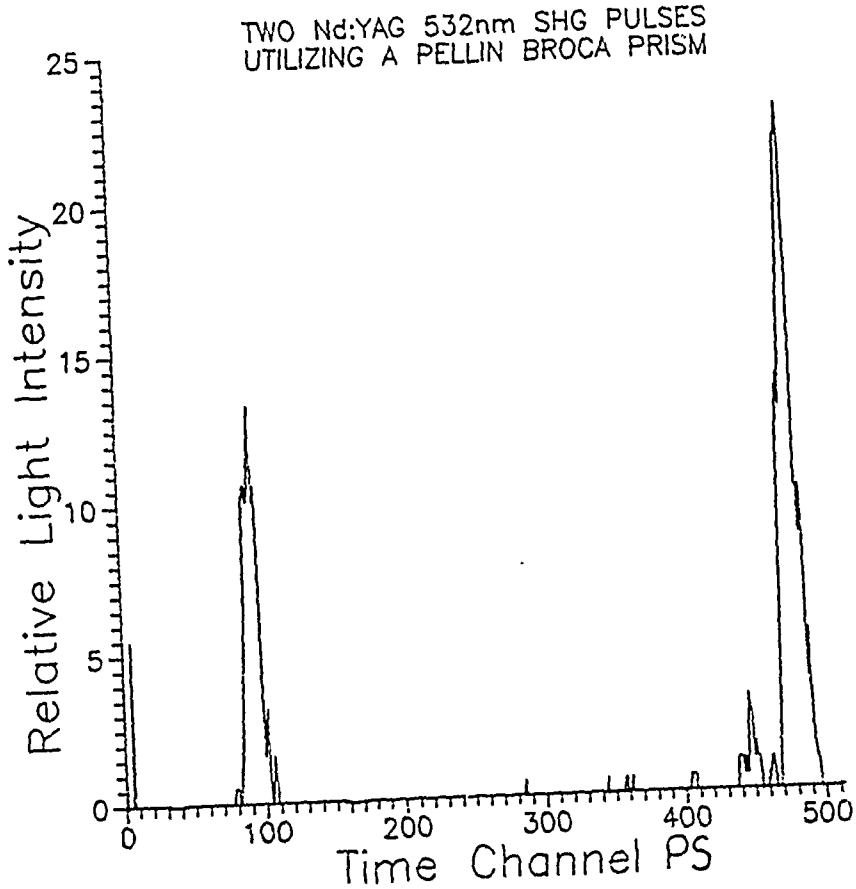
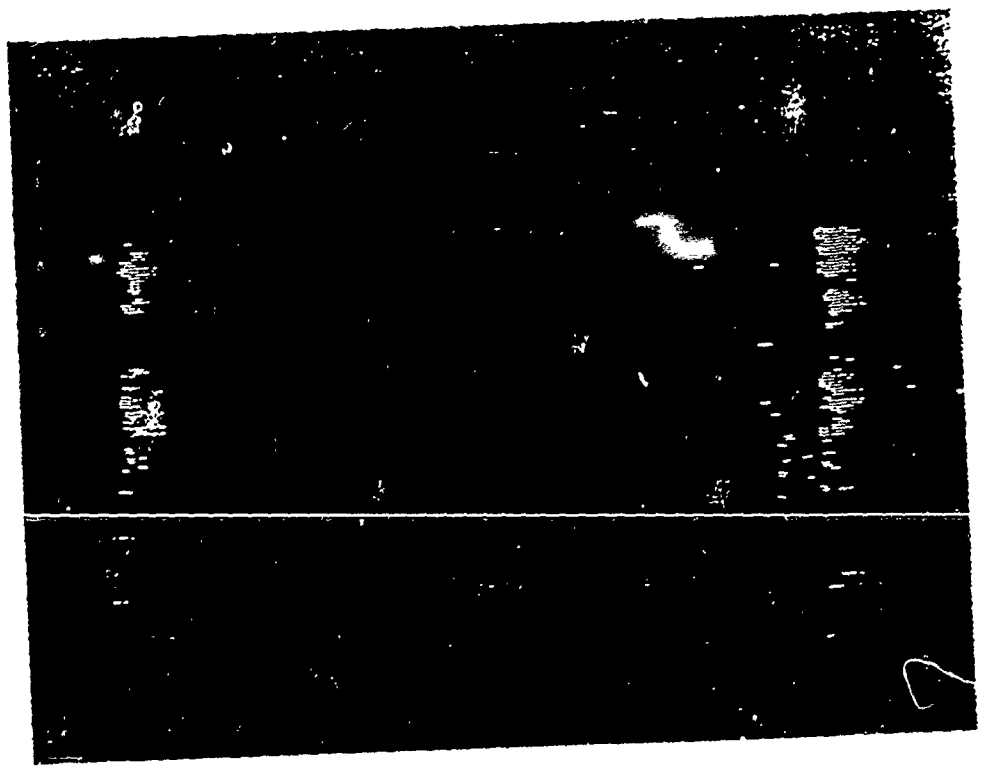


FIGURE 4



PICTURE 4

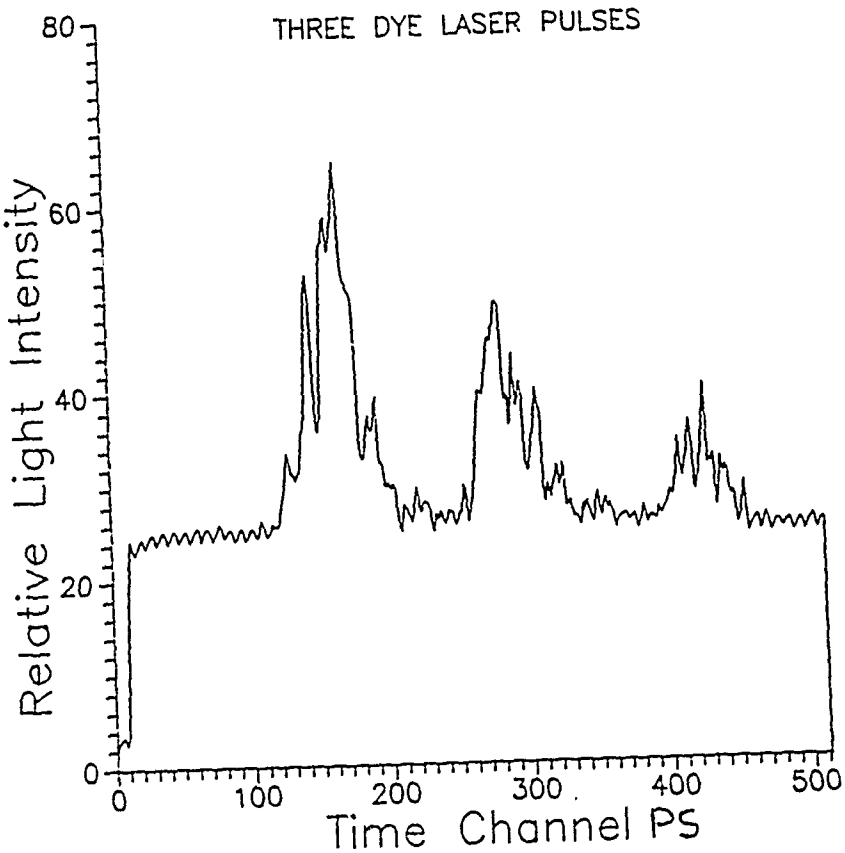
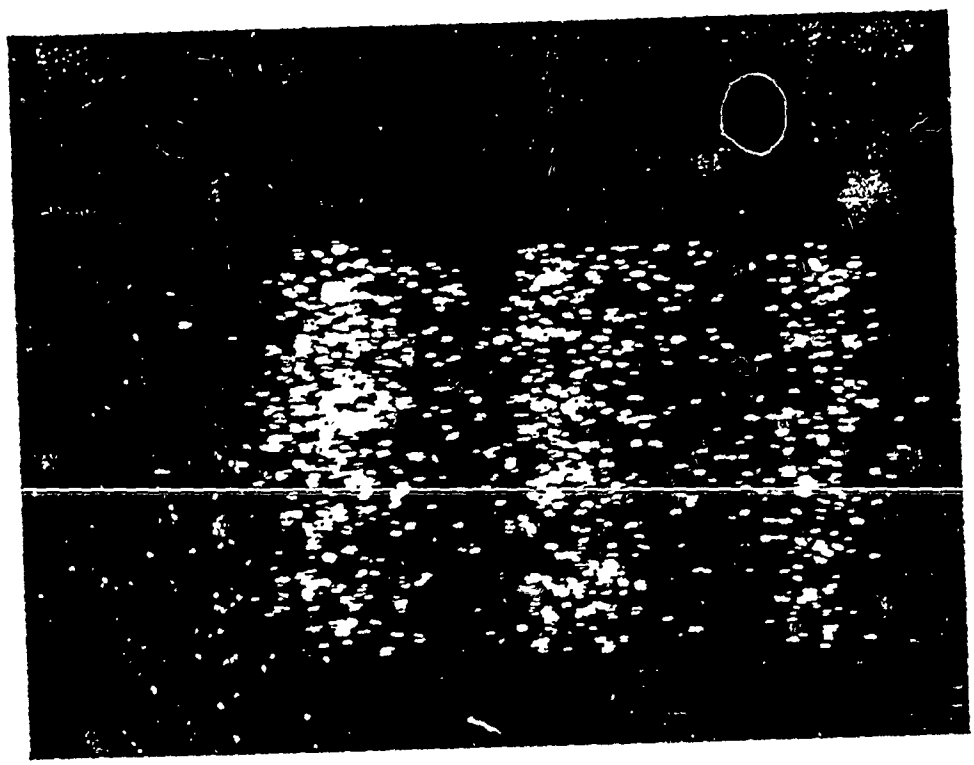


FIGURE 5.



PICTURE 5.

94-15

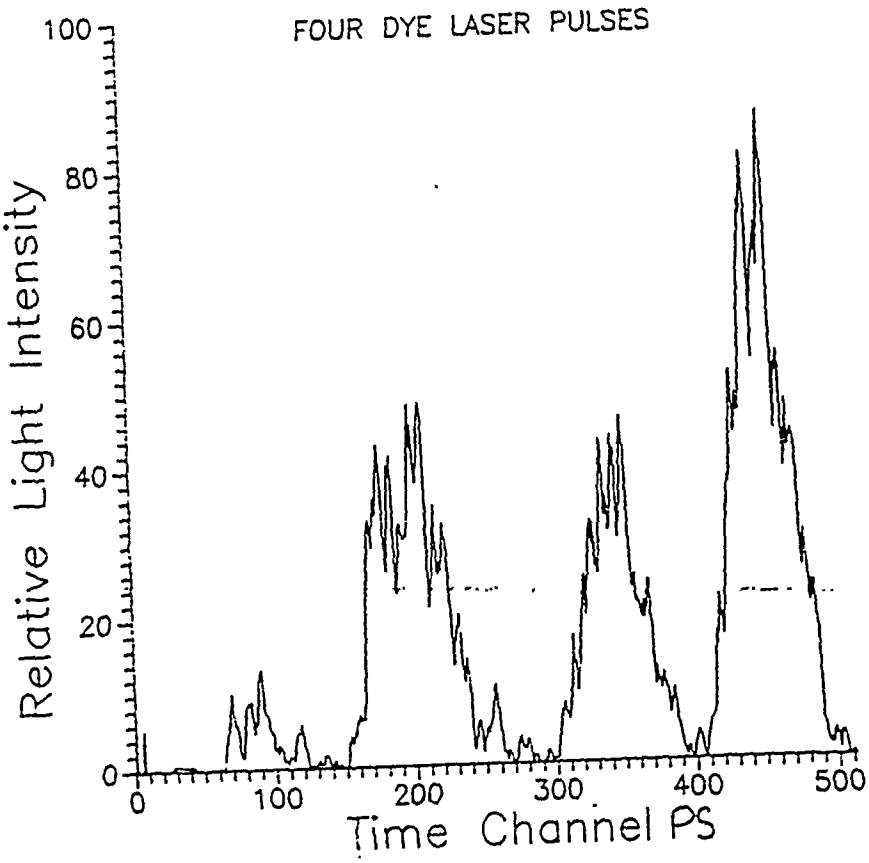
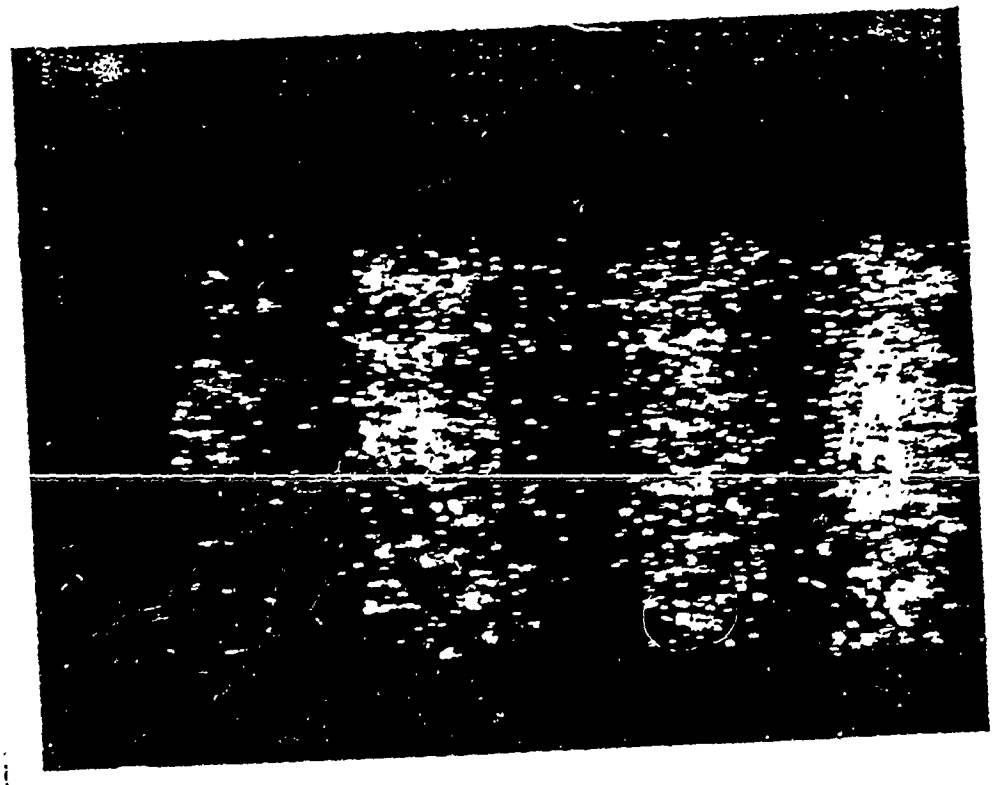


FIGURE 6.



PICTURE 6.

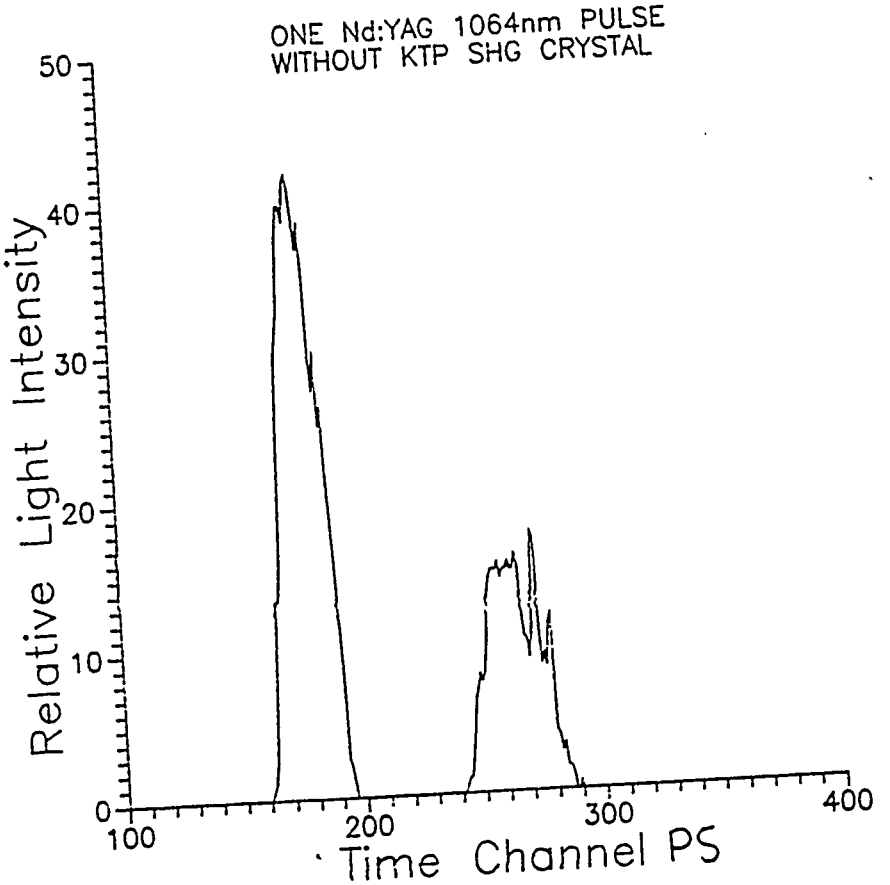
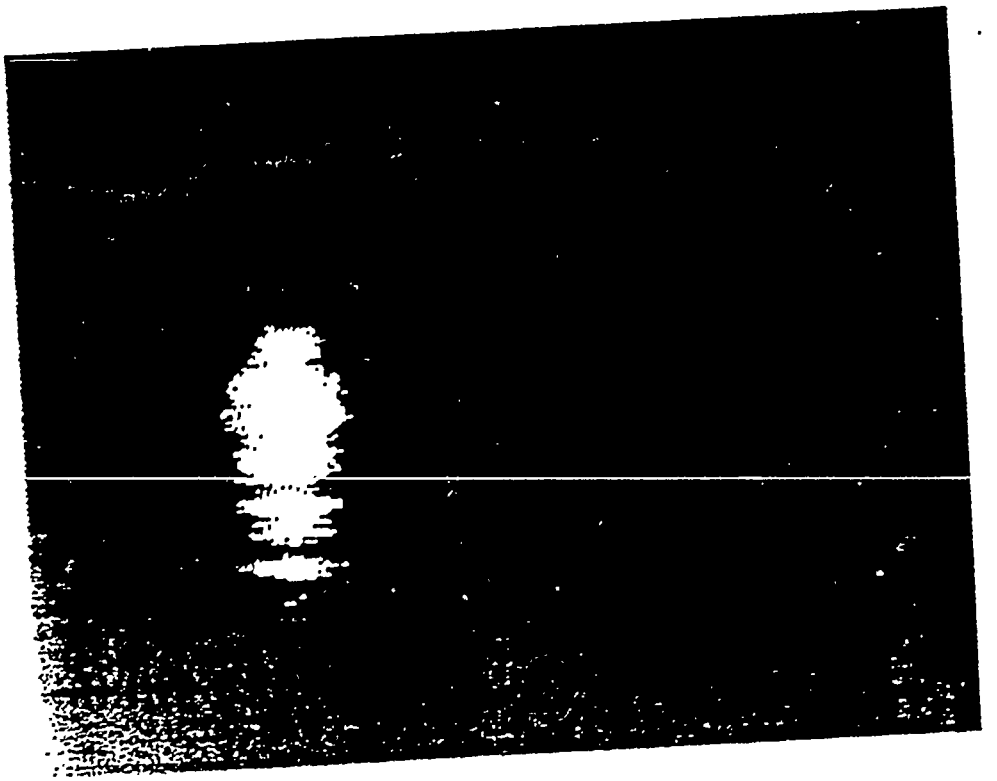


FIGURE 7.



PICTURE 7.

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FINAL REPORT

PCR Analysis and in situ Detection of Ureaplasma urealyticum and Mycoplasma hominis.

Prepared by:	Paul Calvo Vito G. DelVecchio, Ph.D. and Raymond Wolfe
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University:	University of Scranton
Research Location:	USAFSAM/EKLM Brooks AFB TX 78235
USAF Researcher:	Ferne K. McCleskey
Date:	11 August 1989
Contract No:	F49620-88-C-0053

Same Report As
Prof. Vito DelVecchio
(Report # 158)

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FINAL REPORT

GLUTAMATE INVOLVEMENT IN THE PHOTIC ENTRAINMENT OF ACTIVITY RHYTHMS IN HAMSTERS

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Department and University:	School of Medicine Mehary Medical School
Research Location:	USAFSAM/NGNS Brooks AFB San Antonio TX 78235
USAF Researcher:	Michael A. Rea
Date:	October 17, 1989

Glutamate Involvement in the Photic Entrainment of Activity Rhythms in Hamsters

by

Brian A. Davis

ABSTRACT

A preliminary investgation of light-induced phase shifts of the free-running activity rhythm in male Syrian hamsters was conducted to determine the role of excitatory amino acid neurotransmitters in the photic entrainment of the circadian pacemaker. In the first experiment, hamsters were exposed to a 15 minute pulse of 33 lux (n=5), 250 lux (n=5), 2850 lux (n=6) and > 20,000 lux (n=1) of white light to determine the photic stimulation condition which resulted in a sub-maximal phase shift of the free-running activity rhythm. Exposure to a 15 minute pulse of 250 lux of white light resulted in the most consistent phase shifts. However, no significant effect of light intensity on the magnitude of the phase shift was observed. In the second experiment, the effect of microinjections of 0.3 uL of 10 mM kynurenic acid (KA) or artificial CSF (aCSF) vehicle administered directly into the suprachiasmatic nuclei (SCN) via a stereotaxically-implanted guide cannula on the free-running activity rhythm was determined. No significant effect of KA was observed. Unfortunately, we were unable to conduct the final experiment designed to determine if light-induced phase shifts of the pacemaker could be blocked by KA microinjection. The procedures developed during my tenure at the School of Aerospace Medicine provided a founda-tion for future studies of the neurochemistry of photic entrainment of the SCN circadian pacemaker in hamsters.

ACKNOWLEDGEMENTS:

I would like to thank the Air Force Office of Scientific Research, Universal Energy Systems, and the School of Aerospace Medicine for providing me this unique opportunity in Neurochemistry Research.

I must give special thanks to Dr Michael Rea who presented me with challenging and interesting projects throughout my stay. Thanks also to Heather Alexander, Joanna Saucedo, Dr Bob Gannon, Dr Dave Terrian and the other members of the Neurosciences Laboratory for their help, expertise, coffee, and humor. Thanks to Edith Bautista for her help and organization.

INTRODUCTION:

Many members of both the animal and plant kingdoms employ internal timekeeping mechanisms or "pacemakers" to regulate physiological and psychological function. Often these phenomena present in cyclic fashion, yielding infradian, circadian, or ultradian rhythms. Of these, circadian flucuations in physiological and behavioral function are subjectively most apparent. We are acutely aware of our tendency to become sleepy at night and active/alert during the day. Perhaps less apparent our the multitude of physiological rhythms that occur within us and insure that our bodily funtions are properly tuned to the diurnal environment in which we live.

Circadian rhythms are not passive responses to our diurnal environment. Recent data have shown that they are controlled by a pacemaker, or group of pacemakers, which is/are entrained to the the diurnal environment (Rusak and Zucker, 1979). From experimental evidence, it is apparent that sunlight is a major zeitgeber in the natural environment (Moore-Ede, et al, 1982). A zeitgeber is "a periodic factor of the environment able to entrain circadian rhythmicity" (Aschoff, 1960). Although other environmental cues such as food or water availability may also act as zeitgebers, the rising and setting of the sun provide the principle entraining signal responsible for "resetting" the circadian pacemaker in the natural environment.

In mammals, the light-entrainable circadian pacemaker appears to be located in the suprachiasmatic nuclei (SCN) in the hypothalamus (Rusak and Zucker, 1979). The SCN are located on either side of the third ventricle, and immediately dorsal to the optic

chiasm within the hypothalamus. The SCN receive entraining photic information by at least two pathways; a monosynaptic projection from retinal ganglion cells (Lenn and Moore, 1973), the retinohypothalamic tract (RHT), and an indirect retinal projection through the ventral lateral geniculate nucleus (vLGN)/ intergeniculate leaflet (Card and More, 1982), the geniculohypothalamic tract (GHT). The RHT projection appears to be both necessary and sufficient to support photic entrainment of the pacemaker (Rusak and Zucker, 1979). The role of the GHT in photic control of the pacemaker is unclear.

Recent studies by Liou et al (1986) revealed that optic nerve stimulation released ^{3}H-glutamic acid, an excitatory amino acid. It has, therefore, been proposed that glutamate is released from RHT synapses upon photic stimulation and, upon acting on SCN neurons, may be one of the mechanisms responsible for photic entrainment. By measuring changes in behavioral rhyhtms upon photic stimulation, we can quantitate the effect of light on the SCN pacemaker. If glutamate is released from the RHT, and is responsible for photic entrainment of SCN-driven rhythms, microinjection of a glutamate antagonist directly into the SCN should reduce or eliminate the changes associated with photic stimulation.

OBJECTIVES:

A) Determine whether male Albino rats or male Syrian hamsters respond more consistently to light-induced phase shifts.

B) Construct a device which allows us to provide a reproducible photic stimulus to experimental rodents.

C) Determine the photic stimulation conditions which yield a sub-maximal phase shift advance of the free-running activity rhythm.

D) Develop stereotaxic coordinates for microinjections of excitatory amino acid antagonists directly into the SCN.

E) Determine the effects of microinjection of kynurenic acid, a glutamate antagonist, directly into the SCN as a control for future glutamate studies.

EXPERIMENTAL PROCEDURES:

In the first experiment, male, Sprague-Dawley rats were housed in wheel cages and their activity (wheel turns) was monitored using a Zenith 248 computer. For the first 7 days of the experiment, the rats were entrained to a light-dark schedule of 12 hours of light and 12 hours of darkness (LD 12:12) to demonstrate intact photic entrainment pathways. Under LD 12:12, all animals showed circadian activity rhythms entrained to the LD cycle. Next, the rats were maintained under constant dim (<1 lux) red illumination. During this period, animals continued to display activity rhythms which were observed to free run, a phenomenon whereby the endogenous period of the rhythm (pacemaker) is observed (Fig 1).

Each day, a given animal began wheel-running activity slightly earlier or later than on the previous day (Fig 2). The time at which activity began was defined as cir-cadian time 12 (CT12). The average period of the free-running rhythm (Tau) was calculated from the slope of activity onsets obtained over several consecutive days according to equation 1.

$$\text{Tau (hrs)} = 24 + \text{slope of activity onsets} \qquad (1)$$

After 7 - 10 days under constant dim red illumination, the rats were stimulated with a 15 minute pulse of 250 lux of white light at either CT14 or CT18. Stimulation at these times has been reported to cause either phase delays or advances, respectively, of the free-running activity rhythm. Stimulation times were calculated according to equation 2.

$$CT(n) = (CT\ 12_p - Tau/2) + (n/24)\ X\ (Tau) \quad \text{in hours} \qquad (2)$$

Where: CT (n) = Desired CT on day of stimulation

: n = CT time desired

: $CT\ 12_p$ = Projected CT 12 for day of stimulation

We calculated CT 14 and CT 18 for all of our animals and randomly placed them into two groups, one group (n = 5) received a 5 minute pulse of 250 lux of white light at CT 14, the other group (n = 4) received the pulse at CT 18. After the photic stimulation, the animals usually showed a transition period before a free-running period stabilized. Once a free-running period was re-established (about 3 days), the magnitude of the phase shift was determined. The slope and Tau of the days following stimulation were calculated. The first three days after

stimulation are not used in the calculation to avoid the transition period. The slope and Tau prior to stimulation was used to extrapolate to the day following stimulation to determine what CT 12 would have been for that day had we not interfered. This time was compared with CT12 predicted for that same day using slope and Tau following stimulation (Fig. 2). The difference between the CT 12 predicted using data before and after stimulation constitutes the phase shift. If an animal began wheel-running activity earlier than predicted (i.e. without stimulation), a phase advance has occurred. The converse represents a phase delay.

The activity rhythms observed with Sprague-Dawley rats were characterized by short bouts of low amplitude wheel running and unreliable times of activity onset (Fig. 1). This made accurate calculation of CT12 difficult. Furthermore, rats showed great variability in their response to photic stimulation at both CT 14 and CT 18 (Fig. 3). Our data is summarized below:

CT	n	Phase Shift	Phase Shift
14	5	- 1.46 ± 1.33 Hours	- 1 Hour 28 minutes
18	4	- .57 ± .67 Hours	- 34 minutes

Syrian hamsters were tested and found to display much more robust activity rhythms with consistent and predictible times of activity onset (Fig. 2). Therefore, hamsters were used for the remainder of the study.

Sixteen adult male Syrian hasters were introduced to wheel cages and transferred from LD 14:10 to constant dim red light.

Groups of randomly-selected hamsters were exposed to 15 minute pulses of white light at intensities of either 33 lux, 250 lux, 2850 lux or 22000 lux at CT18. Light pulses administered at this time have been reported to induce phase advances of the free-running activity rhythm in hamsters (Takahashi et al., 1984). The light stimulation apparatus was a Vivitar Model 2000AF slide projector, consisting of a 150 W tungsten-halogen lamp (type A1/216), a glass infrared filter, and a series of collimating and projection lenses. Light intensity could be controlled by introducing 5 cm x 5 cm neutral density filters into the slide compartment (Fig 4). The stimulation chamber was white metal cylinder (12 cm dia. X 20 cm) covered with optical diffusion glass. The light intensity inside the chamber was determined using a Tektronics J-16 photometer (J6511 probe). Figure 6 shows the maximum phase advances produced by light stimulation over this fluence range. The data are summarized below and in figure 5.

Fluence Level	n	Average Shift
33 Lux	5	+ 19.6 ± 72.8 mins
250 Lux	5	+ 21.8 ± 45.2
2850 Lux	5	- 47.0 ± 185.0
22,000 Lux	1	376

Although the 250 lux group displayed the most consistent phase shifts, all groups showed consid-erable variability in their response to light stimulation. No significant effect of light intensity on the magnitude of the phase shift was observed.

It is possible that our failure to observe fluence-dependence is due to an effect of the dim red light condition. McCormack and Sontag (1979) reported that very low level red light could entrain activity rhythms in albino rats. This experiment is currently being repeated under conditions of constant total darkness.

Our next task was to establish stereotaxic coordinates for the SCN, so that reliable and reproducible intra-SCN injections of a glutamate antagonist, kynurenic acid, could be made. We began with coordinates of 0.7 mm lateral of the midline, 0.7 mm anterior to the bregma, 7.6 mm ventral to the dura, maintain-ing a five (5) degree angle to the vertical, and positioning of the incisor bar 2.0 mm below the interaural line (Meijer et al, 1988). Methylene blue dye (0.3 ul) was injected into the supra-chiasmatic hypothalamus of hamsters and the location and spread of the dye was determined histologically. These coordinates did not prove to be accurate, and after consulting the literature and the laboratory of a colleague, Dr Elliott Albers, new coordinates were established. These were 1.5 mm lateral of the midline, 1.5 mm anterior to the bregma, 7.7 mm ventral to the dura, maintaining a ten (10) degree angle to the vertical, and positioning of the incisor bar at the interaural line. These coordinates proved accurate, and we began cannula implantation.

Animals were anesthetized with pentobarbitol, until all deep pain reflexes were absent. Two small holes were drilled into the skull, the first aimed at the SCN, the other to support a small set screw. The 26 gauge microinjector delivered dye injections, once again with good results. Seventeen (17) animals received

permanent 26 gauge guide cannulae stereotaxically aimed at the SCN and affixed in place with dental cement. The animals were returned to their activity cages and monitored for 4 days under LD 14:10 followed by 10 - 15 days under constant dim red light to insure that (1) robust, entrainable activity rhythms persisted after surgery, indicating intact retinal afferents, (2) regular free-running rhythms continued after transfer to constant red light, indicating a proper SCN function, and (3) the guide cannulae remained stable and patent. Unfortunately, only nine of the seventeen implanted hamsters qualified for further study. After at least 10 days under constant dim red light, these nine were randomly assigned to two groups, one group (n = 5) received 0.3 ul of 10 mM kynurenic acid (KA) in artificial CSF, the other group (n = 4) received 0.3 ul of artificial CSF vehicle as a control. All animals received injections at CT18, calculated from the free-running activity rhythm as described above. After injection, the hamsters wre allowed to continue under dim red light for at least 10 days and the effect of the microinjection (phase shift) was evaluated as described above. At the end of the experiment, each hamster received a microinjection of 0.3 ul of methylene blue dye and the location of the microinjection was confirmed histologically. Seven of the nine animals showed accurate injections. The results of the seven remaining animals are summarized below:

Compound Injected	n	Average Shift
aCSF	3	- 15.5 $\pm$ 32.2 mins.
KA	4	+ 9.9 $\pm$ 22.0

No significant effect of microinjection of the drug was observed. Therefore, this protocol can be used in the final experiment of the study designed to determine if KA, a glutamic acid antagonist, can block light-induced phase shifts of the circadian activity rhythm in hamsters. Unfortunately, this experiment could not be performed during my tenure at the Neurosciences Laboratory.

RECOMMENDATIONS:

Based on the extreme variability of results in both Part I and Part II of our experiment, I suggest that:

A) We attempt to reproduce the Takahashi group results at CT 14 and CT 18, by using sixty (60) minute light pulses at 250 lux.

B) We duplicate both Part I and Part II of our experiment in the hopes of having a reproducible pattern emerge from the data.

C) We keep open minds with respect to the possible neurochemical mechanisms involved in the photic entrainment of activity rhythms in mammals.

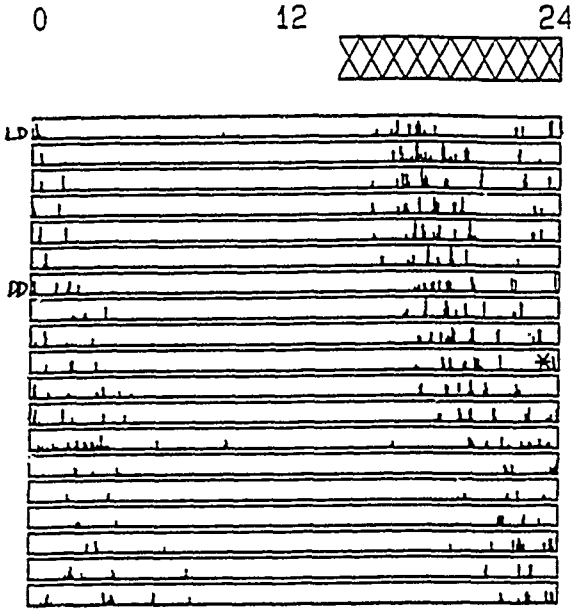

Figure 1

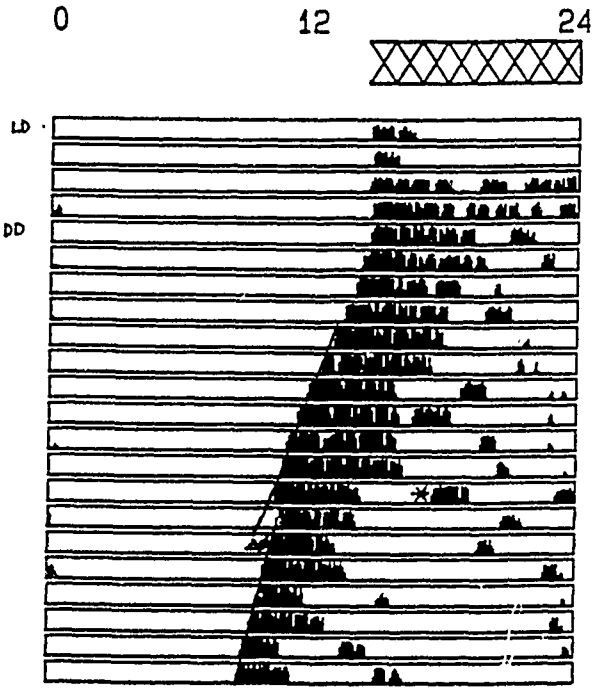

Figure 2

Figure 1. Typical actogram of a male albino rat. Note the sporadic behavior associated with short bouts of activity. The asterisk (*) indicates the day and time of stimulation with a 15 minute burst of 250 lux white light. The crosshatched area represents the time of darkness.

Figure 2. Actogram of Mesocricetus auratus showing how a phase-shift is calculated from daily activity records. The asterisk (*) and crosshatched areas represent the same as before. The $\Delta\phi$ symbol represents the calculated phase shift for this animal.

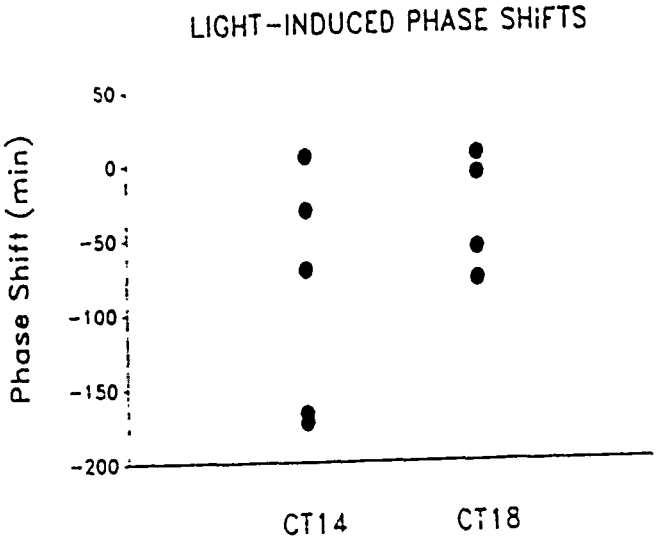

Figure 3

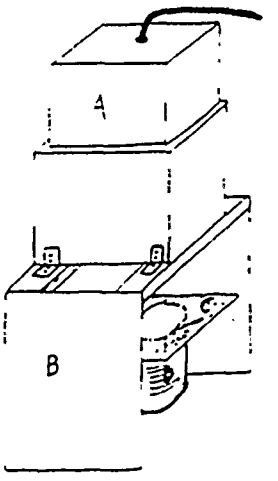

Figure 4

Figure 3. Light-induced phase shifts. Circadian time (CT) plotted against resultant phase shift. Male albino rats were stimulated with a 15 minute burst of 250 lux at CT14 (n=5) and CT18 (n=4). Note the extreme variability in both groups.

Figure 4. Schematic representation of wood housing constructed for photic stimulation of Mesocricetus auratus. (A) is the VIVITAR slide projector; (B) is the projector housing; (C) is the optical diffusion glass; (D) is the white interiored cylinder which contained the animal during the procedure.

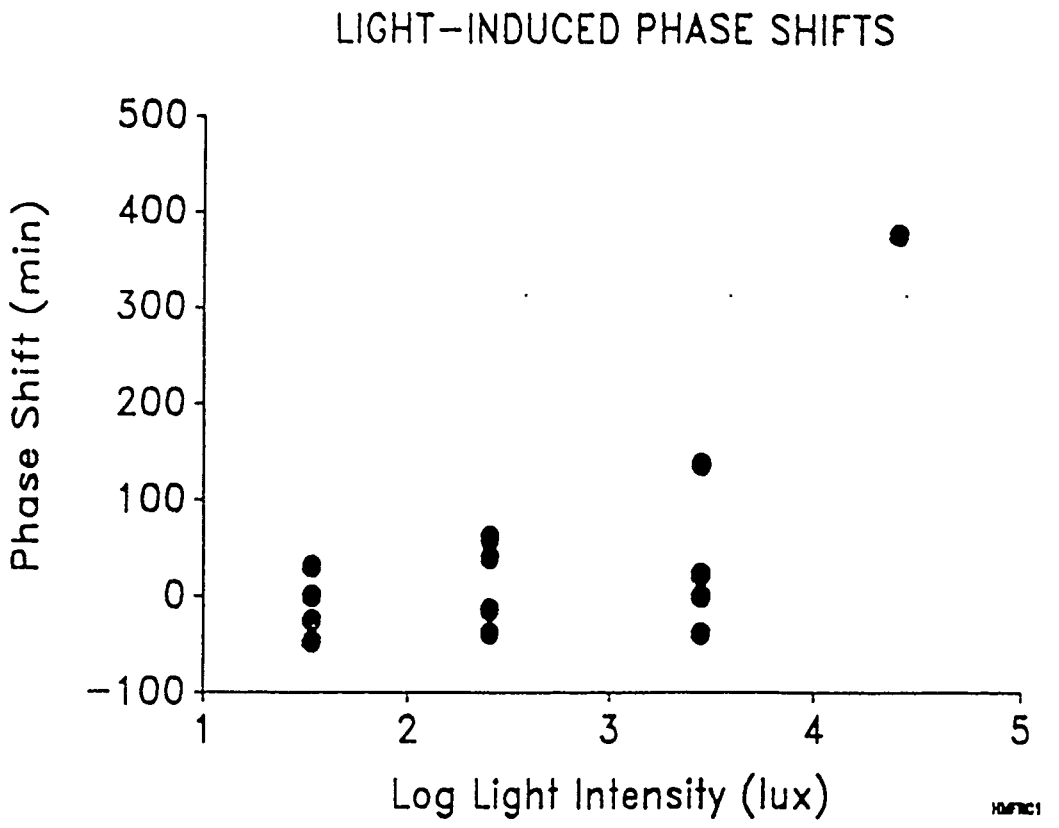

Figure 5. Light-induced phase shifts. Log of light intensity (in lux) plotted against calculated phase shift at 33 (n=5), 250 (n=5), 2850 (n=5), and >20,000 lux. Animals were subjected to five minute bursts of white light at each level on day 10 of DD.

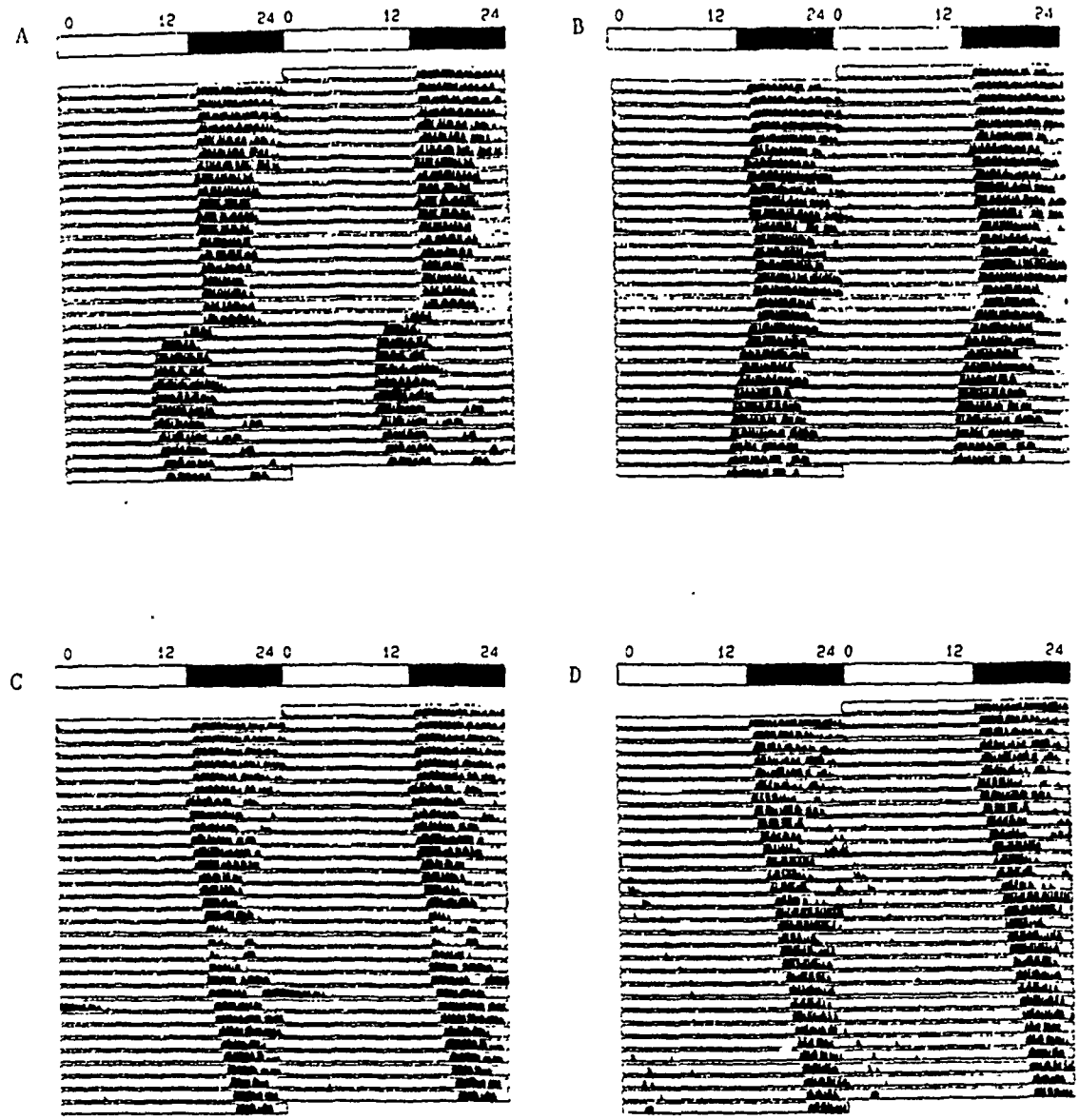

Figure 6. Light-induced phase advances of the circadian activity rhythm. Shown are selected records obtained from hamsters that received 15 minute light pulses at CT18 (*) of white light at intensities of either (A) 22000 lux, (B) 2850 lux, (C) 250 lux, or (D) 33 lux. Details of the activity records are described under Figure 1.

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FINAL REPORT

MAGNETODETECTION BY ANIMALS

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Date:	24 August 1989
Contract No:	F49620-88-C-0053

MAGNETODECTION BY ANIMALS

by

Dagmar C. Fertl

ABSTRACT

A literature review of orientation by animals using the earth's magnetic field was prepared (TP-89-). A photoreceptor-based mechanism, optical pumping, has gained the interest of many scientists. Of particular interest are the biological and physical implications that the blowfly Calliphora vicina possesses this ability. It also appears that magnetic fields intertwine the visual system and the pineal gland. The report discusses the before mentioned and its significance for the medical field and the study of acuity of night vision in pilots.

Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems must be mentioned for their help to me in all administrative aspects of this program.

Dr. John Taboada at Brooks Air Force Base provided ideas for direction and clarification of the project, and was a great help in proofreading and suggesting recommendations for this paper. Dr. Russell Burton's interest in the fulfillment of the research goals allowed for Dr. John Phillips to come to Brooks under the SAM Commanders Lecture Series to lecture about the involvement of visual receptors in magnetodetection.

I also wish to thank Dr. Rex Moyer for the use of work space at Trinity University, and for his assistance in obtaining necessary information for completition of this project. Dr. John Phillips of Indiana University was helpful in his clarification of work being done on magnetodetection. Dr. Mike Rose of Texas A&M University supplied information for the culturing of blowflies. I also thank George Kim, my fellow research student, for his moral support and friendship during the internship.

I. INTRODUCTION:

The visual system has been implicated in the detection of magnetic fields, and appears to be the site of action for magnetic field effects on the pineal gland.

The Biophysics Section of the Clinical Sciences Division of the USAF School of Aerospace Medicine at Brooks Air Force Base is particularly interested in improving night vision in pilots. Of interest is the theorized collaboration of the pineal gland and visual system. Published studies have shown that acuity of night vision in humans responds to magnetic field changes. This has great implications to the Air Force for improving the acuity of night vision in pilots. The division is also interested in developing magnetic field detectors that are smaller in scale than those currently in use. A smaller and more sensitive means of magnetic field detection would be of great use in the field of medicine to map the natural magnetic fields of the body, such as those of the heart.

My research interests are in the field of marine mammalogy, particularly the ecology of cetaceans. It has long been of interest to scientists in the field whether magnetic field detection is involved in the migrational and navigational abilities, and most interesting, the stranding phenomenon.

II. OBJECTIVES OF THE RESEARCH EFFORT:

There has been no recent published review of the studies done on magnetodetection by animals. It has recently come to the attention of the Air Force that the visual system of birds and advanced flies seems to be involved in magnetodetection.

My assignment as a participant in the 1989 Summer Graduate Research Program (GSRP) was to do a literature review of magnetodetection by animals and to determine if J.B. Phillip's work with the visual receptors of the blowfly, which are proposed to be utilized in magnetodetection, should be of interest to the Air Force for funding.

Due to the length of the literature review, Dr. Russell Burton and Dr. John Taboada suggested that the literature review be turned into a technical paper to be published at Brooks Air Force Base. Dr. Taboada also suggested that information concerning magnetic field effects on the pineal gland and involvement of the visual system be added to the literature review.

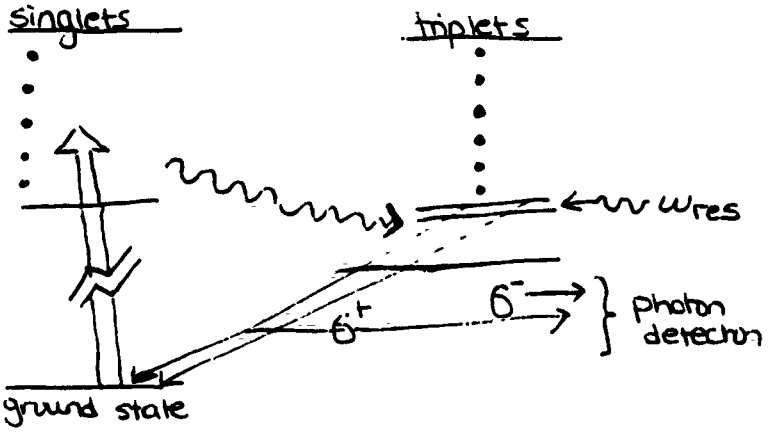
III. MAGNETIC DETECTION AND RETINAL PHOTORECEPTION

The biological significance of magnetic sensitivity in mammals has yet to be established. Two aspects should be considered. One possibility, is that mammals, like many other animals, seem to use magnetic stimuli for orientation (Fertl 1989). Another possibility is that it plays a role in the circadian rhythm cycle (Bliss & Heppner 1976) - the vertebrate time-keeping system.

So far, the only well-explained orientation process based on the earth's magnetic field is the "magnetotactic" behavior of some bacteria and algae. It is based on chains of ferrimagnetic particles inside the organism providing just enough alignment in the organism passively along the field lines. Many animals, such as insects, fish, birds, reptiles, and mammals, have been determined to possess magnetite (see Fertl 1989), however, the significance has yet to be determined in most cases. Demonstrating the presence of magnetizable material is only one step in the experimental procedures necessary to establish that an organism uses magnets to detect the earth's magnetic field and orients relative to it. Anatomical and physiological correlates may be suggestive, but they do not prove the existence of a perceptual ability.

The suggestion that magnetic detection may be a photoreceptor-

based mechanism has been gaining momentum. Leask (1977, 1978) proposed that magnetic field detection in birds takes place in the eye, in the molecules of the retina as an adjunct to the normal processes of vision. This "optical-pumping" mechanism is of interest because it transforms the problem of magnetic moment or field detection into one of photon detection.



The schematic diagram of optical pumping process in a molecule having a singlet (S=0) ground state (as nearly all molecules of biological interest have zero magnetic moment in the ground state) (Leask 1978). Photon absorption is a broad-band process to the singlet excited states. Excitation transfer to triplet states (S=1) is via (anisotropic) spin-orbit interaction, and splittings shown in lowest triplet state are due to spin-spin (dipolar) interactions.

In order to achieve magnetoreception via the triplet, there are a number of requirements as described by Leask (1978): (1) the 3 triplet sublevels must be populated, presumably by excitation transfer from the excited singlet (broadband) levels normally reached by transitions from the singlet ground

state (2) the populations thus achieved must be dissimilar (3) the effect of the geomagnetic (or any other) field on the sublevels must be anisotropic, in order to achieve a directional response (4) there must exist a local well-defined frequency source in the MHz range, which will come into resonance with the triplet sublevels and cause population equalization between them.

The model is directly testable, in that the process requires incident radiation (possibly visible light) and the simple issue to be decided by an experiment is whether or not light is essential to the process of magnetoreception. Being an axial rather than a polar phenomenon, it is consistent with observations that birds do not make use of the polarity of the magnetic field (Wiltschko & Wiltschko 1972). Wiltschko and Wiltschko (1981) conducted experiments with inexperienced young pigeons after transportation in total darkness. They found that while their results were consistent with Leask's hypothesis, it was obvious that transportation in total darkness is another way of preventing the collection of meaningful orientation information during the outward journey to the release site. Semm and Demaine (1986) have provided evidence implicating the visual system of birds in magnetoreception. The retinal photoreceptors are the postulated magnetoreceptors for magnetic compass orientation. Magnetically and light directional selective cells were found

in the stratum griseum et fibrosum superficiale of the optic tectum (visual cortex). Response peaks varied with the orientation of the pigeon in the horizontal plane, confirming the magnetic responses contained directional information.

Leask's theory of optical pumping (1977) has been extended to other animals. It has been proposed by Phillips (1979, 1987, 1989) that flies are able to orient using the magnetic field. He feels that in advanced flies (such as Calliphora and Musca), a subpopulation of the retinula 7 and 8 cells (in the compound eye) are able to discriminate the phosphoretic emission of a single triplet substate of a pteridine pigment(s) present in the visual cells.

Since a large portion of the fly head is the compound eye, these animals are considered to be highly visual. The compound eye is highly complex, consisting of a few thousand ommatidia, each with its own facet lens. Behind each lens, 8 rhabdomeres (photoreceptors) are arranged, such that 8 photoreceptors behind different lenses look into the same direction. The axons of photoreceptors R1-R6 synapse on second order neurons in the lamina, wheras the axons of R7 and R8 pass directly to the medulla (van Hateren 1986). Because of the difference in the pattern of convergence, it has been considered that the R7 and R8 are functionally separate from R1-R6. Studies (e.g. Stark et al. 1977) have shown that the spectral sensitivity profile of R1-R6 photoreceptors exhibits

2 peaks, in the UV and blue-green range, respectively. Retinula 1-6 cells of the blowfly contain an antenna pigment that absorbs near-UV light and transfers energy by a radiationless process to a visual pigment absorbing at longer wavelengths. Phillips (1987) found a weak magnetic effect on energy transfer in R1-6 cells provides a substrate on which natural selection could operate to give rise to the R7/8 magnetoreception system. Under natural conditions, small changes in energy transfer efficiency would not be detectable in R1-6 cells, because of masking due to direct excitation of the photopigment by light.

There are 2 types of R7 and R8 cells. R7(y), R7(p), and R8(p) are proposed to be the 3 separate visual inputs necessary to accurately detect small changes in the efficiency of energy transfer that indicate the relative alignment of the earth's magnetic field (Phillips 1987, 1989). However, in the R7(y) cell, also containing a UV-absorbing antenna pigment, sensitivity of the blue-absorbing photopigment to direct absorption of light is greatly reduced by an optically dense screening pigment. Thus, the R7(y) photopigment is primarily sensitive to energy transfer from the antenna pigment. UV-sensitive R7(p) and blue-sensitive R8(p) cells provide addtional inputs necessary to determine the proportion of UV light absorbed by the R7(y) antenna pigment that is transfered to the photopigment, and to factor out the component of R7(y)

response due to direct excitation of the photopigment by light bypassing the screening pigment.

Pteridines (xanthopterin, isoxanthopterin, and riboflavin) present in receptor cells provide candidate accessory pigments that (a) populate the triplet state with high quantum efficiency (b) exhibit absorption spectra similar to that of the accessory pigment in R7(y) and, (c) emit phosphoretic (triplet state) energy at wavelengths that coincide with the absorption maxima of the photopigment in R7(y).

Phillips has also found that behavioral responses of blowflies to earth-strength magnetic fields are influenced by exposure to radio frequencies in the range from 1 to 5 MHz (Phillips 1987). This effect of radio-frequencies is also a direct prediction of the optical-pumping model suggested to Phillips by Dr. R. Silsbee of Cornell University (Phillips 1989). The flies' magnetic response is even sensitive to the radio frequencies produced by fluorescent lights (Phillips 1989)

It is Phillip's work that has recently come to the attention of the Air Force. The significance of the ability of the blowfly to amplify the extremely small interaction of an earth-strength magnetic field with an organic molecule to the level of photon detection raises a number of interesting issues concerning both the physical and biological processes involved. A magnetodetector on the scale of that present in

the blowfly has great implications for medical science. The Air Force could develop a magnetodetector to map magnetic fields in the human body, such as those in the heart. This process also sheds light on the structure of biological membranes. The influence of natural or artificial magnetic fields on biochemical reactions remains highly controversial. In most cases, the fact that tends to be overlooked is that the thermal energy of the molecules considered sensitive to magnetic fields is much higher than the interaction energy between the magnetic field and the molecules (Cremer-Bartels et al. 1983).

To carry out such a study, culturing techniques and experimental setup must be considered. Information on culturing the blowfly can be obtained from Greenberg & George (1985). Experimental setup should follow that devised by Phillips for his work (1989).

IV. MAGNETIC DETECTION AND THE PINEAL GLAND

The pineal gland of lower vertebrates such as fish, amphibians, and reptiles is primarily a photoreceptor organ and its electrical activity changes in response to environmental lighting (Dodt 1973), whereas the mammalian pineal gland is a secretory organ and no longer responds to direct illumination. The pineal gland in mammals is a

neuroendocrine transducer; it converts neural signals that are generated in the retina by light, and transmitted to by post-ganglionic sympathetic neurons, into a hormonal output, i.e., the secretion of melatonin (Cardinali et al. 1972). The pineal gland's functions and its individual cells have been shown to respond to changes in the ambient magnetic field (see Fertl 1989). Findings suggest that melatonin, a hormone secreted by the pineal gland, plays a role in regulation of light adaptation in the retina. On the basis of the finding that high melatonin content in the retina may be dangerous for the integrity of visual cells in response to light exposure, it seems very important that rapid inhibition of melatonin biosynthesis occurs to protect the retina against blinding. Such inhibition appears to be sensitive to the magnetic field. Pang and Yew (1979) postulated increased night-vision acuity concomitant with increased melatonin content in the retina. This finding should be of great interest to the Air Force in its studies to improve the acuity of night vision in pilots.

Data also points to a retinal magnetosensitivity which may serve to modulate pineal gland function. The site of action for magnetic field effects on the pineal gland in birds has been suggested to be the visual system, particularly the retina (see Fertl 1989). This sensitivity, however, appears to not be limited to birds. The mammalian retina also has been shown to be magnetically sensitive. Cremer-Bartels et al. (1983) found that human night vision acuity is sensitive

to magnetic field variation. Reuss and Olcese (1986) in their work with rats found that stimulation by dim light is necessary for the perception of weak magnetic fields. If one could assume the photoreceptors in the retina to be magnetoreceptors, then the combined presence of both red light and a weak magnetic field might generate sufficient activation of the photoreceptors to give rise to impulse activity in the visual system pathways, thereby leading to an inhibition of pineal function.

V. RECOMMENDATIONS

a). The review points towards a number of Air Force research questions:

1. Is magnetic reception connected to phototransduction in a rhodopsin based vision system?
2. Is the role of melatonin to modulate night vision?
3. Can magnetic detection be tested in a simple phototaxic system, such as the Chlamydomonas reinhardtii algal cell?

b) The review suggests the conduct of experiments on the most simple organism that is phototaxic, see for example prior work on Chlamydomonas reinhardtii. Biochemical endocrinological studies of the pineal gland of mammals (rodents, for example) to clarify the role of light on magnetic field effects are

also suggested.

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FINAL REPORT

Cryopreserving Chlamydomonas reinhardtii at -70°C by the Two-step Cooling Method

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Cryopreserving Chlamydomonas reinhardtii at -70°C by the Two-step Cooling Method

by

George G. Kim

ABSTRACT

A protocol was developed for cryopreserving Chlamydomonas reinhardtii algae, strain 125, by gathering as much information within the ten week period as possible. A storage temperature of -70°C was found to be lethal to the cells. A -196°C storage temperature should provide greater survival. Further experiments, using the final protocol, should produce significant numbers of viable cells surviving after cryopreservation.

Acknowledgements

I thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsoring this research. I thank Universal Energy Systems for their administrative help as well as the activities of which I was involved.

I thank Dr. Taboada for his supervision and support in making this summer run smoothly. The suitable working environment provided by Trinity's biology professor, Dr. Moyer, as well as his support and suggestions were invaluable to this project. The initial protocol as well as some sources given to us by Tom Nerad of American Type Culture Collection provided a valuable starting point. The technical support given to this project by David Gaines was especially appreciated.

I. INTRODUCTION:

The Photobiology Program of the Clinical Sciences Division of the USAF School of Aerospace Medicine at Brooks Air Force Base is currently studying phototaxis in Chlamydomonas reinhardtii algae as a biochemical model of the human eye since rhodopsin has been demonstrated to be an algal photoreceptor pigment as it is in mammalian eyes. Some means were needed to store the algae during periods of relatively little use (i.e. the academic year) using the available equipment. Optimally, a supply of frozen algal cells which could be thawed and used immediately as a source of phototactic cells is a desired goal. This research was performed to find just such a method of cryopreserving the algae.

My previous experience with Chlamydomonas reihardtii, in its maintenance, contributed to my assignment to this particular project. My experience involves the biweekly transfers of the cultures onto new growth media, the preparation of the growth media (High Salt (HS), High Salt with Acetate (HSA)) in different forms (broth, plates, and slants), as well as the cryopreservation of mammalian cells (hamster tumor cells).

II. OBJECTIVES OF THE RESEARCH EFFORT:

Due to the abscence of previous considerations for cryopreserving algae our primary objective was to obtain information on this topic as well as perform a few pilot studies. In order to establish a most feasible method we attempted to preserve the algae Chlamydomonas reinhardtii strain 125 under certain conditions. The algal cultures were grown by incubation at 25°C under constant light conditions between 1800 and 3000 lux (or 500 and 800 uW/cm^2). The growth media used was either High Salt (HS) or High Salt with Acetate (HSA). The algal cultures were passed to new media plates with the appropriate broth overlay every 48 to 72 hrs.

As a standard for quantifying our results from freeze storing the algae, plated colony counts were performed for viability counts. To perform a plate colony count, a serial dilution of the harvest of sample from the algae was made. A small volume of the dilutions were streaked onto a HSA plate and incubated under growth conditions for light and temperature mentioned above, for over seven days. The colonieswere then counted, and based upon the dilution and volume used for the streak plate, the original concentration of cells were calculated.

The original goals which became non-feasible during this research period was to determine any morphological changes induced by the cryopreservation technique. A Gilford spectrophotometer was to be used to test the phototaxic ability of the cryopreserved generation's

filial generation. After the first experiment it was clear we needed to develop a technique which provided viability before any tests on morphology could be done. Total counts by the use of the Coulter Counter became useless information, although many counts were made along with a few cell size distributions, due to the fact that no viable cells could be produced from the three experiments performed. Therefore we relied on the plate counts for information pertinent to this paper.

III. Experiment 1.

a. The first information was obtained for cryopreserving algae from Tom Nerad of American Type Culture Collection (ATCC). The process outlined involved making a sterile 10% methanol in growth medium solution. This mixture was added v/v to the freshly harvested algal cells to give a 5% MeOH/growth medium. The algal cells were to be either in late log phase or early stationary phase. This algal preparation was then aliquoted into 2 ml quantities in Eppendorf tubes to be frozen at a rate of 1°C/min to -40°C and then quickly frozen to liquid nitrogen temperature of -196°C. Upon thawing, the cell suspension was to be diluted with growth media to eliminate the harmful effects of MeOH. We performed the protocol with a few changes. The rate of cooling was about -2.9°C/min. for the sample (See fig. 1 (Δ)) down to the -70°C temperature, instead of -40°C, at which the samples were stored. In thawing the cells the Eppendorf tubes were vigorously shaken in a hot water bath at 52°C (except when otherwise stated) until the last ice crystal disappeared. The tubes were centrifuged in an Eppendorf

centrifuge for ten seconds. The supernatants were discarded and the cells were resuspended with one milliliter or HSA. The growth media used was HS which is more effective at producing phototaxis in algae than HSA.[18] The age of the algal culture was two days old which according to a previous experiment by Moyer et. al. indicated that the cells were in the period of greatest phototaxic ability.[18]

The algae was grown and frozen in HS medium since HS endowed better phototaxic ability on the algal cells than HSA.[18] The original goal was to test for phototaxic ability, of the filial generation of the cryopreserved specimens, to determine if there existed any morphological changes during the process.

b. The original harvested concentration of cells was 1.52×10^7 cells/ml. After two days at -70°C the cells were thawed and its viability counts indicated no growth. Another sample vial was thawed five days post-freeze and its contents were poured onto an HS plate with an HS broth overlay. This was to be used as a phototaxis assay. This plate had growth of algae with a fungal contaminant so it was restreaked for purification purposes. A purified colony was placed onto an HSA slant with the HSA broth overlay. Due to technical difficulties (research assistant autoclaving the cells) the cell line was lost. A last vial was obtained and was thawed at 35°C and the media was diluted out by centrifugation, and resuspended in HSA broth onto a HSA plate. The result was no growth.

Results: 0% viability

The 35°C water bath was used because the thawing temperature should not be greater than the upper limits of biological tolerance.[12]

IV. Experiment 2.

a. Three sources were suggested by Tom Nerad of ATCC.[16, 17,15] These three sources reference G. J. Morris[16,17] work and were used to modify the previous protocol. According to Morris, mthanol was the only cryoprotective agent of any effectiveness for cryopreservation of Euglena gracilis at -196°C. Morris also found an effective general method for cryopreserving algae by the two-step method of cooling.[17] This method required the temperature of the cells to be dropped to -30°C and held at that temperature for 15 min and thereafter rapidly reduced to -196°C. The adapted protocol for the second experiment involved harvesting the cells, adding 10% MeOH (v/v) in HSA to the harvest, aliquoting into 1 ml quantities allowing a equilibration time of five min,[17] then plunging the cells into -28°C for 15 min, and then plunging the cels to -70°C. HSA was used because it supports cell growth in hte dark whereas HS does not. Viable counts were performed on the fresh harvest, and on a vial of cells after the 15 min holding period at -28°C and on the cells in a vial stored for five days at -70°C.

The thawing process used was agitation in a 35°C water bath until the last ice crystal had melted. The vials were then centrifuged in an Eppendorf centrifuge for about five seconds. The supernatant was

discarded and 0.5 ml of HSA was used to resuspend the cells.

b. Results of experiment 2

Viable counts- concentrations

harvest: 1.6×10^7 cells/ml

5 days at -70°C: 0 cells/ml

V. Experiment 3.

a. Because the previous experiment's viable count following a five day period at -70°C indicated no surviving cells, and that a viable count following -28°C storage for 15 min needed to be done, the previous experiment was repeated to obtain a viable count after holding the cells at -28°C for 15 min as well as viable counts at 24 hr intervals at holding temperatures of -28°C and -70°C. This was done to obtain information on the rate of cell killing.

b. Results of Experiment 3.

Viable Counts

harvest: 4.14×10^5 cells/ml

15 min at -28°C: 3.54×10^5 cells/ml

24 hrs. at -70°C: 0% survival 24 hrs. at -28°C: 0% survival

VI. Experiment 4.

a. This last experiment is a compilation of all the pertinent informa-

tion essential for the cryopreservation of the Chlamydomonas reinhardtii. First, the two step cooling method is the most readily performable protocol because it doesn't require an expensive apparatus for controlling the freezing rate of the cells. This method was first utilized by Luyet and Keane.[6] This protocol is the best procedure based on the literature surveyed. Due to the lack of time this protocol could not be performed exactly.

The protocol:

The optimal age of the algal cells for freezing is seven days in late log phase of growth, (Morris), in mid-log phase of growth according to Gresshoff,[3] and seven day-old cultures according to Hwang et. al.[5] This discrepancy between Hwang, Morris vs. Gresshoff may be resolved due to algal activity rather than on the phase of growth as stated by Gilboa and Ben-Amotz.[12] Morris obtained a survival of 56% of cells frozen at -196°C[14] whereas the percent survival obtained by Gresshoff was only 7% for Chlamydomonas reinhardtii strain 137c^{+}. Therefore, seven day-old cultures should be used for cryopreservation.

According to the Morris protocol the algae were placed under 1000 lux of light with a 14 hr. light: 10 hr. dark cycle. With our setup, the algae are under constant light of a range between 1850 and 2450 lux. We were not able to assess whether these differences were important or not.

Cryopreservative:

Morris found that 2.5 M methanol (10%) was the most effective cryopreservative for the algal cells.[14] Accordingly, a harvest of algal

cells was prepared and admixed with sterile 20% methanol solution in HSA. After 5 min. of equilibration, the cell suspension was aliquoted into 1 ml volumes in Eppendorf tubes. The vials were cooled to -10°C seeding temperature and held there using an ethanol bath at -10°C of 125 ml for seven minutes to allow the solution to freeze according to Leibo's protocol[2] referenced by Morris.[14] Next the vials were dropped to -30°C holding temperature and held about 48.6minutes to acclimitize the cells.[14] After that the cells should be plunged to -196°C and stored. The thawing process should involve agitation of the sample in a 35°C water bath.

Step	Schematic of Ideal Protocol	References
1	Culture and harvest seven day old algae.	14, 5
2	Add 20% MeOH/HSA v/v to harvest and equilibrate for five minutes, aliquot in 1 ml volumes.	14
3	Uncontrolled drop (see fig 2) the vials to -10°C without a bath, and hold at seeding temperature for seven minutes.	7, 14
4	Uncontrolled drop the vials to -30°C, hold for 48.6 minutes.	14
5	Uncontrolled drop the vials to -196°C (liquid nitrogen temperature storage.	14
6	Thaw by agitation in 35°C water bath til the last crystals have melted.	12, 14
7	Centrifuge and discard supernatant, resuspend in .5 ml HSA.	

Due to limited time the final experiment was changed at a couple of steps: At step 1 we used 5 day old algae; at step 5 we cooled the vials to -70°C rather than -196°C.

b. Results of Experiment 4.

Viability counts in concentrations

Original harvest concentration: 3.69×10^7 cells/ml

Post -28°C holding temp 15 min: 2.13×10^7 cells/ml: 58% recovery

Post -28°C holding 24 hrs: 0% recovery

Post -70°C freeze (1 day): 7.00×10^3 cells/ml: .019% recovery

Post -70°C freeze (2 days): 1.00×10^3 cells/ml: .0027% recovery

Post -70°C freeze (over 3 days): 0.00% survival

Speculation: A -196°C storage temperature should produce a much greater percentage of surviving cells. Morris obtained about 50% survival rate.

VI. RECOMMENDATIONS:

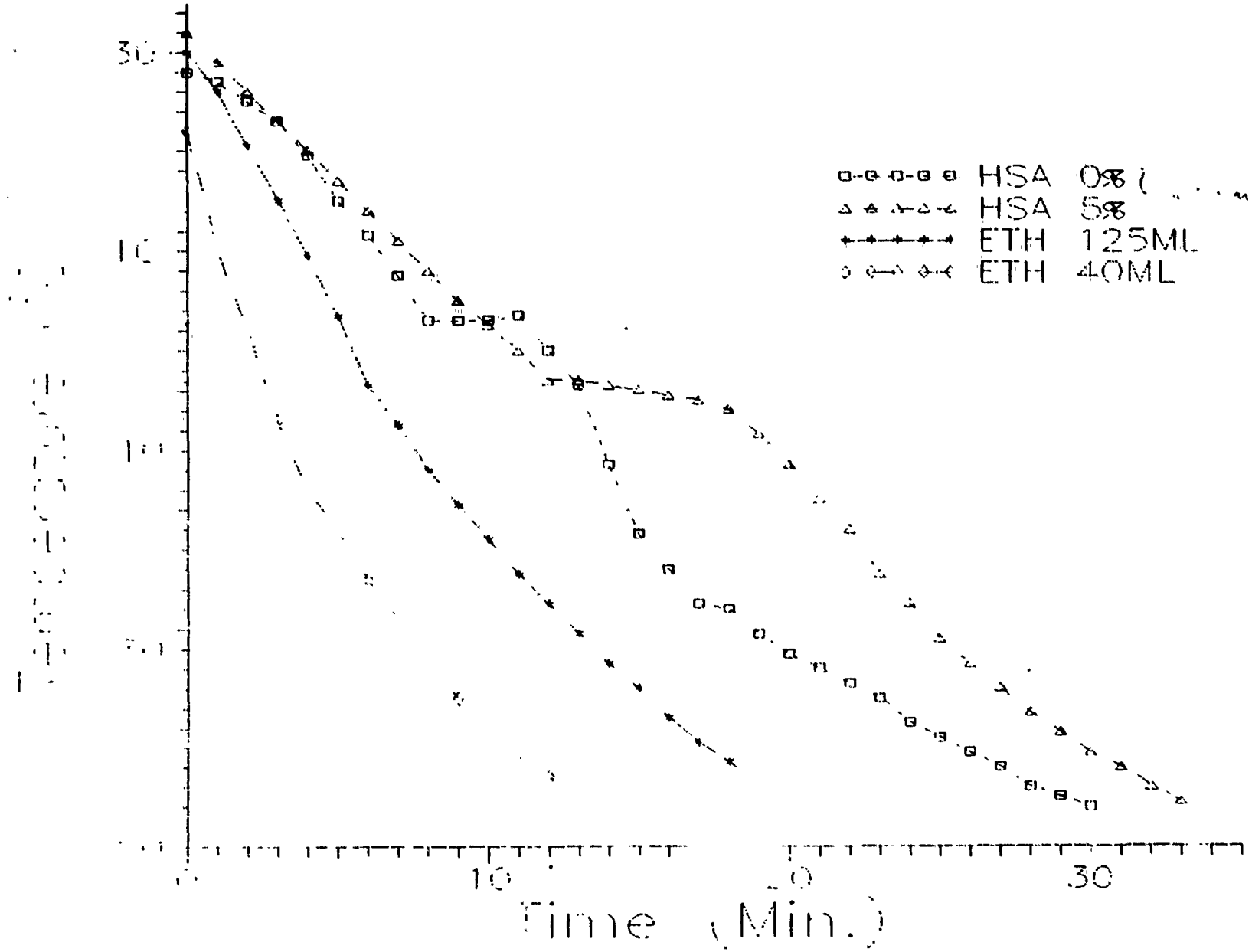
a. The procedures for cryopreserving algae must take into account methods for eliminating the lethal effects of freezing. Peter Mazur has expounded on the two major causes of lethality in cells.[9,10] The damage to cells due to freezing is mainly caused by intracellular ice damage or by extracellular solute concentration buildup. If the rate of cooling is too slow, extracellular ice formation occurs by crystalizing in a relatively pure water molecule network, forcing electrolytes into concentrated pockets along with the cells which

kills them. On the other hand, if the cells were frozen too quickly the solutes would not pool but would be frozen in the liquid. The danger would then arise from the formation of intracellular ice. The primary site of damage occurs at the cellular membrane as evidenced by studies on mitochondria and chloroplasts.[5] Cell membranes lose their ability to block the passage of ice crystals below - 10°C to - 15°C and perish due to intracellular ice damaging the membranes and causing the cells to become leaky. Cells normally do not freeze internally above -10°C. Super cooled water has a higher vapor pressure than ice, therefore, at 0°C to -10°C the membranes equilibrate with their external environment by losing water and thereby the cells become dehydrated which lowers the intracellular aqueous vapor pressure. Therefore, one must establish conditions which are intermediate between those of slow and rapid cooling. Cryopreservative agents help to alleviate the damages by the opposing forces. Molecules of low cellular permeability such as glycerol, dimethylsulfoxide (DMSO) and methanol prevent an increase in intracellular electrolytes during the freezing process. Nonpermeable macromolecules such as polyvinylpyrrolidone (PVP), polyethylene glycol, serum albumin, and dextran, protect by increasing the freezing point depression of the solution by preferentially binding water molecules which reduces the amount of free water available which results in an increase in solute concentration. Methanol also increases the degree of unsaturation of phospholipid fatty acids,[6] and affects membrane fluidity,[4] adding to the cryopreservative effect. According to Mazur, quick freeze produces small ice crystals which can, even at -100°C, recrystallize and reorganize and expand resulting in membrane

rupture. To prevent this from occuring a quick thawing process is essential. and a storage temperature or less than -196°C.

The final experimental protocol takes all or these factors into account and should be the optimal procedure ror cryopreservin, Chlamydomonas reinhardtii. One must keep in mind the temperature limits ror growth and reproduction are low (about -30°C)[13] and photobiological excitation extinctions are much lower (-1°K).[1]

b. A more indepth study of this procedure should be done to determine its effectiveness using the storage temperature at -196°C since -70°C proved too ineffectual. Also tests for morphological changes after freezing needs to be done using phototaxis of the parental's filial generation as the criterion. Previous studies indicate that Chlamydomonas cells are stable at -196°C[9] and do not induce morphological changes.[11]



98-15

Fig. 1. Cooling curves of 1 ml samples of HSA (0% MeOH) (□), and an HSA (5% MeOH) (△), in a 125 ml ethanol bath. Cooling curves of an 125 ml (✱) and a 40 ml (◇) ethanol baths.

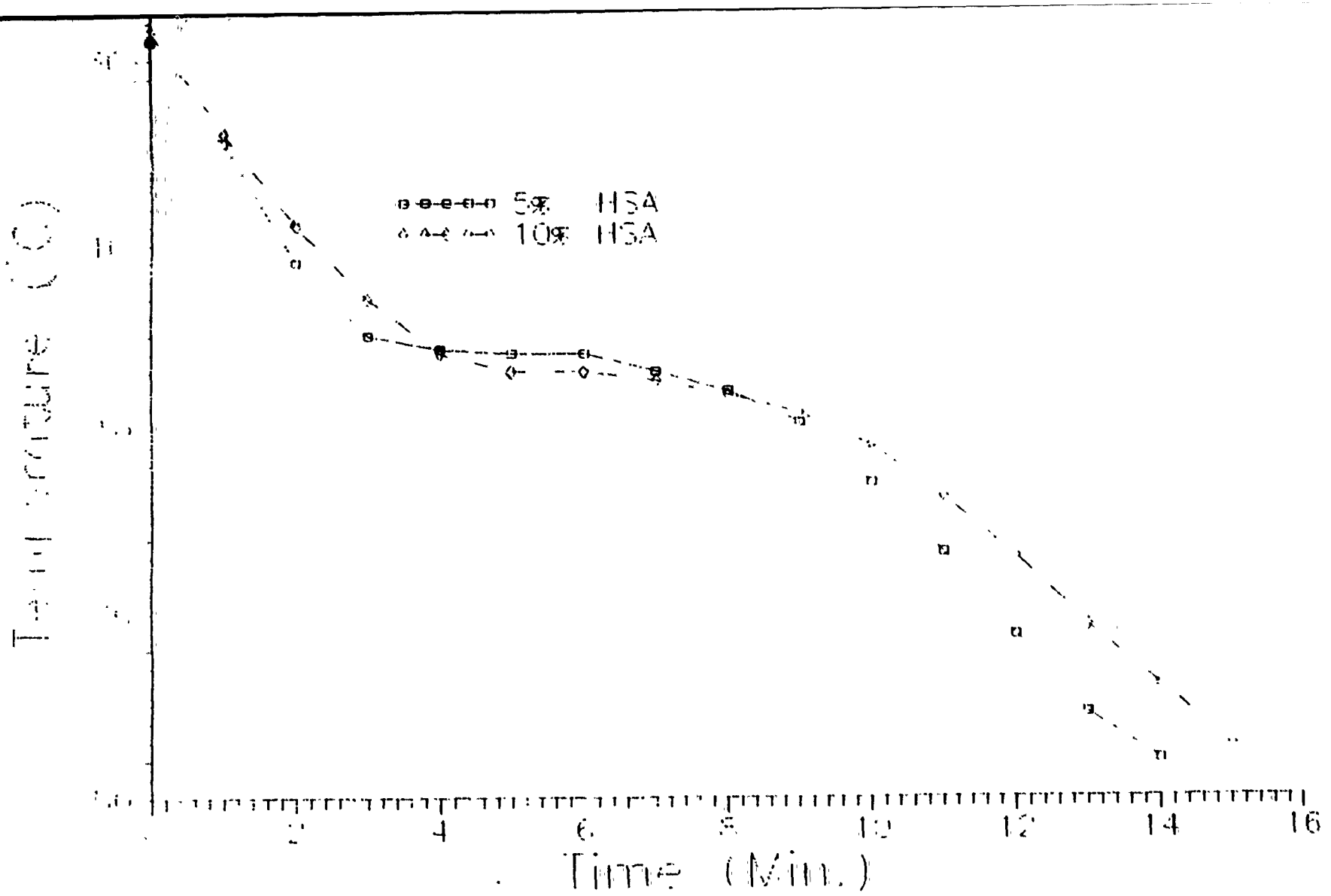


Fig. 2. Cooling curves of one milliliter samples of HSA (5% MeOH) and HSA (10% MeOH), without an alcohol bath, in a -70°C environment.

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FINAL REPORT

STATISTICAL MODELS IN SOCIAL DYNAMICS

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STATISTICAL MODELS IN SOCIAL DYNAMICS

by

Teresa Lee

ABSTRACT

Sociological theories become increasingly concerned with social changes. It is not enough to address the social phenomena merely from either the static point of view or the cross-sectional analysis. Sociologists move on to analyze the actual time paths of change in attributes of individuals and/or societies. While studying statistical models in social dynamics, I concentrate my research on the event history analysis.

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I. INTRODUCTION

An event consists of some qualitative change at a specific point in time. The best way to study events and their causes is to collect the event history data. An event history is a longitudinal record of when events happened to a sample of individuals or collectivities. So they are ideal for studying the cause of events. Event history possesses two features:

(1) censoring(incomplete observation)

(2) time-varying explanatory variables

that create major difficulties for standard statistical procedures.

In the study of recidivism reported by Rossi (1980), 430 inmates released from Maryland state prisons were followed for one year after their release. The events of interest were arrests; the aim was to determine how the probability of an arrest depends on several explanatory variables.

Rossi(1980) simply created a dummy(1,0) variable indicating whether or not an individual was arrested at any time during the 12-month follow-up period. However, dichotomizing the dependent variable is arbitrary and wastes information. It is arbitrary because there was nothing special about the 12-month dividing line except that the study ended at that point. It wastes information because it ignores the variation on either side of the dividing line.

To avoid these difficulties, it is tempting to use the length of time from release to first arrest as the dependent variable in a multiple regression. But this strategy poses new problems of

large biases. (Tuma-Hannan 1978).

Even if none of the observations were censored, one still face another problem: how to include explanatory variables that change in value over the observation period? There is simply no satisfactory way of incorporating time-varying explanatory variables in a multiple regression predicting time of an event. It is increasingly common to find the longitudinal data set with measurements of many variables at regular intervals. For most events, such data are essential to get accurate estimates of the effect on those variables that change over time.

My research interest is to understand models and methods available for analyzing the event history data in sociology. During the past 10 weeks, I learned to use the statistical packages of SAS and BMDP as a tool while analyzing the data. I have run and replicated the same results from the following procedures: ANOVA(Analysis of Variance), REG(Regression), CATMOD(Categorical Data Modeling), GLM(General Linear Models), Correlation and Regression, Hypothesis Testing, Repeated Measures Designs, Multiple Regression Analysis.

II. OBJECTIVES OF THE RESEARCH EFFORT

It is not surprising that modern quantitative methodology in sociology is static -- it was developed in a period in which static images of social structure dominated American sociology. However, theoretical development have shifted toward a greater concern with the processes of social change. American sociologists renewed attention to Marx in the late 1960s has been a major force on promoting the development of theories that stress on changes and its historical context. Theoretical

developments on social movements have also begun to emphasize social dynamics. Tilly(1975) and Skocpol(1979) have been especially prominent in redirecting their attention to the organizational bases of collective protest and revolution. For example, a key theoretical problem is to understand how changes in the strength of contending groups, the repressive power of a state, and the nature of the relations of a state to its neighbors affect collective violence, and social revolution. Understanding the timing of collective protest and its changing forms requires the dynamic analysis. Because of the theoretical trend, interst in explaining how and why social actors and social systems change over time seems to be gaining momentum. If movement continue in this direction, the need for dynamic models and methods will grow.

III. AN OVERVIEW OF EVENT HISTORY ANALYSIS

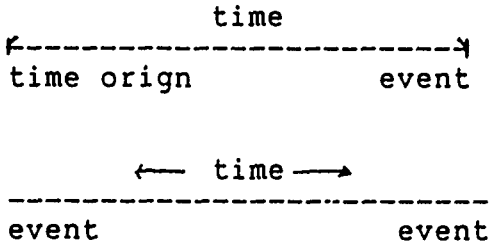
3.1 Methods of Estimation

(A)Life Table: The earliest, best-known, and still widely used method for analysing event history data.

(B)Survival or Lifetime Analysis: The more modern method. The experimenter observes how long the animal survive under each of the toxic treatment.

(C)Reliability Analysis or Failure time Analysis.

Distributional versus Regression Methods: much of the early work on event history analysis can be described as the study of the distribution of the time until an event or the time between events.



Repeated versus Nonrepeated Events:

death, failure time of industrial components ------- nonrepeated event

job changes, marriage --------- repeated event

Single versus Multiple Kinds of Events:

single { A study of job termination may not distinguish one such termination from another

For example:A life table may treat all death alike.

multiple kinds:

A study of job termination $\begin{cases} \text{voluntary} \\ \text{involuntary} \end{cases}$

Death $\begin{cases} \text{due to cancer} \\ \text{due to other causes} \end{cases}$

Parametric versus Nonparametric Methods:

A major bridge between these two approaches is the proportional hazards model of Cox:

$$h(t) = \frac{f(t)}{S(t)}$$

$$F(t) = Pr(T \leq t)$$

$$f(t) = F'(t)$$

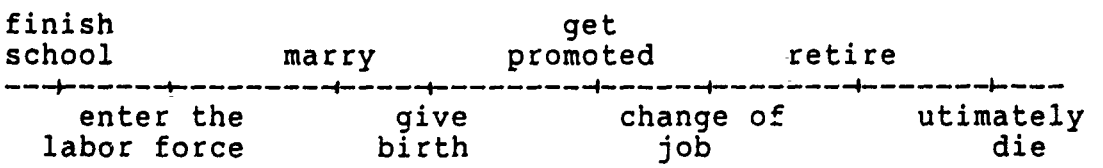
$$S(t) = Pr(T > t) = 1 - F(t)$$

Discrete versus Continuous Time:

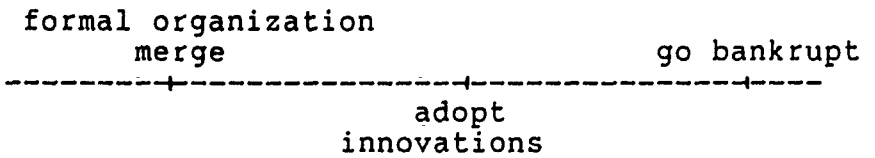
In practice, time is always measured in discrete units.

Discrete-time methods for the Analysis of event Histories:

(1)The history of an individual



(2)The history of an group, organization



(3)The history of nations

experience war		peaceful change of government
	revolutions	

These are characterized as a sequence of events.

When these discrete units are very small, it is viewed as measured on a continuous scale.

When time units are large, it is appropriate to use discrete-time methods which are also known as grouped-data methods.

3.2 Models of Event Histories

(A)Binomial and Multinomial Models:

Binomial ------ when there are two states.

Multinomial ---- when there are three or more states.

Given the wide use of these models in the discrete-time analyses, their continuous-time analyses appear to be very promising for the event- history analysis.

(B)Markov Models:

One way of stating the key assumption of a Markov model is that the instantaneous rate of a transition from state $j \equiv y_{n-1}$ to state $k \equiv y_n$ at a given time t depends only on j, k, and t -- and not on the entire previous history w_{n-1} :

$$r_{jk}(t \mid \omega_{n-1}) = r_{jk}(t), \qquad k \neq j.$$

Markov models also forbid self-transitions: $r_{jj}(t) = 0$.

Applications of Markov models in social sciences

usually make the additional assumption that instantaneous transition rates are stationary in time, i.e.:

$$r_{jk}(t) = r_{jk}$$

A simple conceptual device called operational time permits an elementary form of time variation in transition rates to be retained along with the mathematical simplicity of a time-stationary Markov process. Operational time is useful in dealing with the age dependence of transition rates because of a wide variety of individual behaviors appears to occur with declining frequency as age increases. For example, while aging, people seem to become less likely both to marry and to divorce. On the other hand, operational time is less useful in handling variation in transition rates over historical time because some rates seem to increase whereas others decrease. For instance, in recent decades marital dissolution rates have risen dramatically while rates of marriage have stayed about the same or declined.

(C)Semi-Markov Models:

It is similar to Markov Model in the aspect that transition rates are independent of previous history, while different on the aspects including

(i)Semi-Markov models allow transition rates to depend on the duration in a state.

(ii)Semi-Markov models also permit successive states to be the same.

Through the device of operational time, one can construct semi-Markov models that account for both duration and age dependence

in transition rates. For most social applications, semi-Markov models are more realistic than time-stationary Markov models.

3.3 An Example:Entrance into the Academic Career

According to the normative structure of science proposed by Merton, positions in the academic stratification system should be allocated universalistically based upon his/her contributions to the body of scientific knowledge. Analysis based on the population of male biochemists who obtained their doctorates in the fiscal years 1957, 1958, 1962 and 1963. From this population of Ph.Ds two groups are chosen:

(1) nonmovers: 134 biochemists;

(2) movers: 47 biochemists.

The measure of departmental prestige is based on the study of Roose and Andersen(1970). Productivity was measured using counts of both publications and citations to them. Chemical Abstracts(1955-1973) was used to locate the articles. Citations to these articles, as found in the Science Citation Index (Institute for Scientific Information, Vols.1961, 1964, 1966, 1968, 1970, 1972, and 1974), were used for constructing the citation measures. For details, see Long (1978).

The analysis show, however, preemployment productivity has an insignificant effect on the prestige of the scientists' first academic job.

From Table 1 of Long (1978), Equation 1 regresses departmental prestige on characteristics of the scientists' training and productivity. The productivity measures are based only on work completed before the scientist obtained his first job. Thus the correlation between productivity and position, .096 for publication, and .204 for citations cannot be the results of a departmental effect. The standardized regression coefficients from publications and citations are small and statistically insignificant while those from the prestige of the sponsoring department, the mentor's eminence, and the selectivity of the baccalaureate institution are strong, positive and statistically significant.

From Table 3 of Long (1978), Equations 3 and 4 show that the departmental prestige significantly influences the scientists' prodictivity after the third year in a department. In equation 3 we find that the departmental location is the strongest factor explaining the scientists' level of publications. Thus change in the publication rate is most strongly affected by the prestige of the scientists' academic location -- scientists in prestigious departments increase their publication rate while those in less prestigious departments begins to publish relatively less. The effects of the prestige of the doctoral origin on the level of publications and on the change in the publication rate increase substantially.

IV RECOMMENDATION

I plan to continue my study on the event history analysis at WIU for the next year under Dr. Lee's guidance. I want to learn more about statistical models and methods which are useful in the field of Social Dynamics. In order to achieve my goal, I plan to collect some real data sets in the area of event history analysis.

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FINAL REPORT

A RESEARCH OPPORTUNITY AT BROOKS AIR FORCE BASE; A MULTI-FACETED EXPERIENCE

Prepared by:	Cynthia L. Moorhead
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Research Location:	USAFSAM/VNB Brooks AFB San Antonio, TX 78235
USAF Researcher:	Patty A. Boll
Date:	7 August, 1989
Contract No:	F49620-88-C-0053

A RESEARCH OPPORTUNITY AT BROOKS AIR FORCE BASE; A MULTI-FACETED EXPERIENCE

by

Cynthia L. Moorhead

ABSTRACT

The following paper is a brief report on the various projects this researcher was associated with over the summer research period. My primary project assignment was the isolation of catecholamines from blood plasma of test subjects obtained during the tyrosine study conducted at Brooks AFB over the period of September to December 1988. Mechanical difficulties encountered in the laboratory during my research assignment prevented further work on the catecholamine analysis. To date, this portion of the tyrosine study remains incomplete. Therefore, this researcher had the opportunity to participate in several other projects being conducted at the Crew Technology Division of USAFSAM.

ACKNOWLEDGEMENTS

I would like to thank the Air Force Office of Scientific Research and Universal Energy Systems, Inc. for the sponsorship of this summer program. I sincerely appreciate the opportunity to have worked in a highly diversified research setting. The professional associations and overall exposure to research was extremely stimulating and motivating. The experience that I gained is invaluable.

I would like to give special thanks to my immediate supervisor Patty Boll for creating a very relaxed and enjoyable environment to work in. In addition I would like to thank Dr. Paul Werchan for allowing me the opportunity to work in his laboratory assisting Nancy Dietz on her summer research project.

Special thanks are extended to Dr. Russell Burton for coordinating the summer program at the School of Aerospace Medicine. His organization of weekly seminars and "brown bag" lunches provided an excellent opportunity to meet with other researchers and discuss their projects. In addition I would like to thank the VNB staff for all of their help and the other summer program participants for making the summer a very fulfilling and enjoyable experience.

I. INTRODUCTION

The Crew Performance Division of USAFSAM in conjunction with Massachusetts Institute of Technology conducted a study at Brooks AFB to determine the effects of oral tyrosine administration on plasma catecholamine concentrations. Catecholamines are neurotransmitters that are produced and consumed under stress "fight or flight" situations. The objective of the study is to determine if oral administration of the catecholamine precursor tyrosine will provide an increase in plasma catecholamine concentrations. This increase may convey an increased ability to cope and perform in a stress environment. Test subjects were evaluated for changes in performance on a flight simulator and blood samples were taken for catecholamine analysis. To date, the performance portion of the study has been completed. The analysis of the blood plasma concentrations of the catecholamines: dopamine, norepinephrine, and epinephrine remains incomplete.

This researcher was selected to assist in running the samples through an HPLC (High Performance Liquid Chromatograph) to determine catecholamine concentrations. I am a first year medical student and have recently completed 35 hours of post-baccalaureate undergraduate chemistry, biology and physics courses in which I obtained extensive lab experience. My working knowledge of lab techniques contributed to my assignment to the biochemistry lab.

II. OBJECTIVES OF THE RESEARCH EFFORT

The primary goal of the research effort was to establish a technique to isolate catecholamines from blood plasma and to apply this technique to the plasma obtained from the test subjects. A secondary goal for the summer was to gain broad exposure to the variety of research projects being conducted at the Crew Technology Division. Supplementally, I wanted to become familiar with possibilities of participating and contributing to future research efforts. In addition to these goals I wanted to learn about aerospace medicine and physiology, and the role of the Air Force Flight Surgeon.

III.

At the onset of my appointment I began to work immediately in the biochemistry laboratory. The intermediate goal in working towards isolating catecholamines was to establish a standard curve for the analysis. Standard solutions of the catecholamines norepinephrine, epinephrine, dopamine and isoproterenol were prepared in deionized and filtrated water to make 1000 pg/ml, 500 pg/ml, 100 pg/ml and 50 pg/ml solutions. To each dilution an antioxidant and anticoagulant were added. The next step was to place the standard solutions on the HPLC and to see if they eluted through the column. All stock solutions eluted at the 1000 pg/ml concentration. However, none eluted at lower concentrations. Work continued to determine at which electric potential the 1000 pg/ml standard solutions eluted

with the largest and most distinguishable peaks. An electric potential of .2 volts was found to be optimal for this study.

Next, several attempts were made over the course of the research period without success to elute the catecholamines at a lower picogram concentration. After a review of many papers involving plasma catecholamine isolation a more acidic mobile phase was tried. The previous mobile phase which gave results at high picogram levels was an acetate-citrate buffer with a pH of 5.2. A 10% methanol mobile phase was unsuccessful.

In addition, a more acid preparation of the stock solutions was attempted and resulted in no elution of the lower picogram concentrations. To date, no further progress has been made. The study was slowed down tremendously by many interruptions over the summer. The main in-house air supply went down and the HPLC pump had several pressure and seal problems arise.

IV.

Due to the many mechanical problems encountered in the biochemistry laboratory I had the opportunity to fully participate in several other research projects. The first project I worked on was scoring POMS (Perception of Mood State) and memory tests administered to the Temazepan Study test subjects. I was exposed to protocol formation and test design. In addition, a large portion of my research appointment was spent working on a project with Summer Fellow Nancy Dietz and her supervisor Dr. Paul Werchan.

I assisted Nancy in her surgical procedures on rats to mimic Gravity Induced Loss of Consciousness (GLOC). In this study the vertebral arteries were cauterized and one of the carotid arteries was blocked off. A balloon occluder was placed around the other carotid artery to control the flow of blood supply to the Circle of Willis. EEG, ECG, systemic blood pressure, and Circle of Willis pressure data was taken. I assisted in the data collection and assimilation.

I also participated as a test subject for several other studies being conducted. One of these was a performance study for the AWACS crews. An additional study concerned spatial disorientation based on visual stimulus. My involvement in each of these studies contributed to the positive and enjoyable experience I obtained from my research appointment.

V.

In addition to my broad research exposure, I attended the summer Health Profession Scholarship Program for the Air Force medical students. This included attending lectures on the consulting services of the School of Aerospace Medicine and an in-depth introduction to aerospace physiology and medicine. The mechanical and physiological effects of altitude to include decompression sickness, spatial disorientation, acceleration and GLOC were presented.

VI. RECOMMENDATIONS

This researcher thoroughly enjoyed the exposure to a multitude of research projects. This type of experience is extremely helpful to students, especially high school students, who are interested in science but may not have considered research as a career choice. For myself, this experience helped to confirm in my mind that I do want to be involved in research in the future and that the military offers many opportunities. My recommendation is that the Office of Scientific Research should continue the summer research program. By providing opportunities to explore research career paths young minds may be encouraged to pursue research goals.

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Final Report

System and Signal Analysis of VEP Data and Joystick Error Analysis

Prepared by:	Harold Longbotham, Joe Rea, and Lionel Ramos
Academic Rank:	Assistant Professor, graduate student, graduate student
Department and University:	Division of Engineering University of Texas at San Antonio
Research Location:	USAF/SAM/RZV Brooks AFB San Antonio, Tx 78235
USAF Researcher:	Captain Norman Barsalou
Date:	August 28,1989
Contract No:	F49620-88-C-0053

Same Report As
Prof. Harold Longbotham
(Report # 164)

1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM
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FINAL REPORT

PCR Analysis and in situ Detection of Ureaplasma urealyticum and Mycoplasma hominis.

Prepared by:	Paul Calvo Vito G. DelVecchio, Ph.D. and Raymond Wolfe
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USAF Researcher:	Ferne K. McCleskey
Date:	11 August 1989
Contract No:	F49620-88-C-0053

Same Report As
Prof. Vito DelVecchio
(Report # 158)

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GRADUATE STUDENT RESEARCH PROGRAM

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FINAL REPORT

DENTAL MATERIALS

Prepared by: Terrance Jorden, B.S.

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Department and School of Dentistry

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Research Lunn Dental Clinic/WHMC

location: Lackland AFB

San Antonio, Texas 78236

USAF Researcher: Col John C. Burgess, D.D.S.

Date: 10 August 1989

Contract: F49620-88-C-0053

DENTAL MATERIALS

by

Terrance Jorden

ABSTRACT

The fluoride leach of seven commercial liners and bases was investigated. Two liners which contain calcium hydroxide and are polymerized by visible light, (Prisma VLC Dycal) and Cavalite (Kerr), were compared with a capsulated "Bonded-base" cement (ESPE Ketac-Bond applicap) and two glass ionomer lining cements: Shofu lining cement and XR ionomer lining cement. Timeline, Vitrabond, and Ketac bonding cement were also used.

The strength of different types of pin-retained amalgams was investigated. Stainless steel pins: Stabilok small diameter, TPS, TMS minum, DENLOK, TMS Link Series (Whaledent) and one SS goldplated TMS Minum (Whaledent) were compared with 3 titanium pins; Filpin, Stabilok 6mm and TMS Link Plus.

A pinpull was also conducted with the use of the Instron testing machine. The project consisted of testing the retention in dentin of eight different pins.

ACKNOWLEDGEMENTS

I wish to thank the United States Air Force and the Benjamin Dunn Dental Clinic Research Division for sponsoring this research. Universal Energy Systems, Inc. must be mentioned for their concern and help to me in all administrative and directional aspects of this program- with special thanks to Ms. Debbie Withers.

My experience was rewarding and enriching because of the many helpful, cordial and dedicated people. Dr. John O. Burgess, Dr. Charlie Heartsfield and Dr. Jim Summitt, provided me with support, encouragement, understanding and a truly enjoyable working atmosphere. The help of Drs. Burgess, Heartsfield and Summitt was invaluable in overcoming many technical barriers. Their concerns and untiring efforts were greatly appreciated.

INTRODUCTION:

Prisma VLC Dycal (Caulk), Cavalite (Kerr), (ESPE) Ketac-bond applicap, Shofu lining cement, XR ionomer cement, Timeline, Vitrabond, Ketac bond. are used as base and lining agents. These products have anticariogenic properties through the slow release of fluoride from the restored portions of the tooth to surrounding tooth structure. Teeth normally consist of hydroxyapatite crystals. After a carious lesion is removed and prior to the placement of a restortion, one of these products is inserted. The fluoride released from these base materials replaces the hydroxide and forms fluorapatite crystals, thus creating a much stronger tooth.

Dental amalgam is often used to replace lost tooth structure. When large amounts of tooth structure is missing amalgam pins are inserted into the dentin to retain the amalgam restoration. Stainless steel and titanium pins are commonly used in this procedure. With the addition of these pins, the amalgam lasts longer and better resists tensile and compressive forces.

I am a sophomore dental student at Meharry Medical

College, School of Dentistry. Currently, I rank in the upper 10% of the class of 1992. During the first year of my matriculation at Meharry Medical College, I was taught about various materials, products and instruments, used in the field of dentistry. I also received valuable clinical and laboratory experience. The General Dentistry and Dental Research Department at the Benjamin Dunn Dental Clinic are quite diversified and provide additional practical training in dental health care delivery.

After receiving a Bachelor of Science Degree from Grambling State University, I entered Meharry Medical College(The School of Dentistry). The knowledge I gained during my first year of dental school help me to interact smoothly with the on-going research in the above mentioned department.

II. OBJECTIVES:

a. To examine the resistance form of amalgam restorations retained with 10 different types of pins.

b. To determine the shear bond strength of liners and bases.

c. To determine the fluoride release of liners and bases.

d.To determine the pin retention strengh of eight different types of pins in tooth structure.

III.

a. Extracted mandibular molars, both caries and

restoration free, were selected then measured at the cemento-enamel junction. The measurements ranged from 9-12 mm. The specimens were stored in tap water except during preparation and testing. The occlusal surface of each tooth was reduced with a Torit Model Trimmer to provide a flat surface approximately 1 mm above the cemento-enamel junction. Two hundred and ten mandibular teeth were prepared in this investigation. The roots of each tooth were notched for better retention and embedded in an aluminum mounting ring filled with orthodontic self-curing acrylic. Six pin holes were drilled approximately 0.5 mm inside the dentino-enamel junction of each tooth. Six 0.6 mm self-threading pins were inserted in each tooth following manufacturers instructions. After insertion, the pins were measured and cut 2 mm above the flattened tooth surface. Copalite (Plastodent cavity lining) was evenly applied on the flattened tooth surface as a sealant. A Tofflemeyer matrix band and Moyco Copper bands were secured in place and dental amalgam was condensed into these retainers using hand and pneumatic instruments. The amalgam height was measured distally, mesially, buccally and lingually. The amalgam crown was built to a height of 4mm, 3mm, and 2mm.

Presently, the first 40 specimens are being tested with an Instron Testing Machine (Instron Corp.). A test load and a constant crosshead speed of 0.05" (1.27 mm) per minute is applied until the amalgam fractures. The results will be recorded on an analysis chart.

D. M. assignment as a participant in the 1989 Summer Graduate Research Program (SGRP) was to determine the bonding strength of five commercially available liners and bases. Prisma VLC Dycal (Caulk), Cavalite (Kerr), Ketac bond "bonded base" applicap (ESPE), Shofu lining cement and GC lining cement (GC).

Seventy five extracted maxillary molars were reduced on their buccal surface to expose the dentin. A Torit model trimmer was used to develop a smooth flat surface. A small piece of teflon tape, with a 3mm hole punched in it, was attached to the flatten surface. A very small plastic tube (7 mm height, 3mm diameter) was placed over the hole, while the lining/base material was slowly inserted inside the tube's cavity. The teeth were separated in five sets of fifteen teeth per set, corresponding with the five different materials to be tested.

The specimens were then embedded in aluminum mounting rings filled with orthodontic self-curing acrylic resin. Mounting these specimens required patience and delicate handling, because the material very easily detached from the tooth. The specimens were stored in tap water except during testing. Initially, the retention of the liner/base material to the prepared dentin held quite firmly. However, water storage reduced the adhesiveness. In fact, several specimens dislodged from the teeth prior to testing. Approximately 5 teeth per group were tested by the Instron Testing machine (Instron Corp.). The results obtained were invalid due to

the low number of specimens and the inconsistent range of recorded values. It was decided that the specimens be replaced. Specimens were re-prepared and an immediate shear bond strength performed.

c. The liner/base materials mentioned above are also used to determine the amount of fluoride being released. These materials were applied into the cavity of a small plastic tube (7mm height, 3 mm diameter) and allowed to harden. The hardened product was removed and placed in a plastic test tube. Here again, fifteen specimens per material were formulated. One millimeter of Type I distilled water was added to each test tube. The liner/base material will be stored in 2ml of distilled water for seven days. On the seventh day, the millivolts will be recorded for each material. After which 100 and 200 microliters of Tisab buffer solution will be added and read accordingly. Sgt. David Goddu, USAF Medical Technologist, constructed a curve to convert millivolts to p.p.m. Therefore, the fluoride released will be measured in p.p.m.

d. Sound extracted third molar teeth will be used in this study. Forty-five teeth teeth will be selected according to size so that no tooth has a buccal lingual diameter two mm greater than another. The crowns will be ground flat on a model trimmer, the roots notched, and the teeth embebbed in Caulk Orthodontic Resin (L.D. Caulk, Medford, Delware). Six TMS Minim pins (Whaledent Inc, NY,NY) will be placed in each toot using a slow speed handpiece to drill the holes. The

pins will be inserted with a handwrench. Each drill will be used no more than 20 times before it is discarded. The teeth will then be divided into three groups depending upon the amalgam extending above the cut pins. All pins will be measured and cut so that the pin protrudes two mm above the pulpal floor. A copper band matrix will be adapted to the tooth so will be supported with green stick compound and Dispesalloy(Jonnson and Johnson) amalgam will be condensed mechanically the top is reached. After setting for 24 hours the copper band will be removed and any excess amalgam trimmed from the CEJ. The amalgam will then be trimmed to 2mm above the enamel-amalgam junction for the specimens in group one, the amalgam in group teo will be trimmed three mm above that junction, and the third group will be trimmed to an amalgam height of three mm.

After water storage for one week, the teeth will then be placed into a fixture positioning the specimen at a 45 degree angle to a handpiece. A 45 degree bevel will be placed on the occlusal surface of the amalgam. The specimen will be placed into a fixture designed to hold the pin retained amalgam at a 45 degree angle to the blunt blade of the Instron (Instron, Canton, Mass). Filter paper will be placed over the amalgam restoration to distribute the load applied by the Instron. The load will be applied at 5 mm/min until failure occurs. The peak load at failure will b e recorded in newtons.

IV. RECOMMENDATIONS:

A. The use of Ketac bond is recommended, because it leached the most fluoride.

B. The Link SS Pin is recommended, because it has the best retention strengh in dentin.

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Final Report

"Temperature Effects on Erythrocyte Sedimentation Rates in Whole Blood and on Erythrocyte and Platelet Volumes"

Prepared by:	W. Drost-Hansen, Mag. Scient. (Ph.D)
Academic Rank:	Professor
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Affiliation:	University of Miami School of Medicine
USAF Researcher:	Lt. Col. Wayne R. Patterson, Ph.D.
Date:	September, 1989
Contract Number:	F 49620-88-C-0053

Same Report As
Prof. Walter Drost-Hansen
(Report # 170)

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FINAL REPORT

Collecting Data and Occurrence of AIDS-Related Symptoms: Longitudinal Study of HIV U.S. Air Force Personnel

Prepared by: Paula Mellon, M.A.

Research Location: WILFORD HALL MEDICAL CENTER/SGHMN
Lackland AFB
San Antonio TX 78236-5300

USAF Researcher: Col. Douglas W. Marshall, M.D.

Date: 30 Sep 89

Contract No.: F49620-85-C-0013

Collecting Data and Occurrence of AIDS-Related Symptoms: Longitudinal Study of HIV U.S.Air Force Personnel

by

Paula Mellon

ABSTRACT

The U.S. Air Force screens all in-coming personnel and all symptomatic personnel for acquired immunodeficiency virus infection. Data from sero-positive personnel become part of the on-going longitudinal study which now contains more than 800 hundred cases. Data from successive in-patient admissions were obtained, reviewed, arranged chronologically, and entered into the data bank for future analysis. Data were entered into three mostly-discrete sub-studies: (1) spinal fluid immunoglobulin and electro-physiological determination of normal or abnormal neurological status (124cases); (2) neuropsychologists' yearly reevaluation test scores on a neuropsychological test battery then correlated with yearly re-samplings of cerebrospinal fluid (98 cases); (3) in-patient AIDS Service narrative summary dates and clinical course of opportunistic in-fections which meet Center for Disease Control definitions for AIDS-related symptoms (116 cases). The importance of attaining accurate data and the im-portance of this large and non-self-selected group of cases in clucida-ting accurate Life Table analysis and in promulgating future AIDS research is noted.

ACKNOWLEDGMENTS

I wish to thank the Wilford Hall USAF Medical Center and the Air Force Office of Scientific Research for sponsorship of this research assistantship. Universal Energy Systems must be thanked for support and encouragement to me despite my lack of academic tenure at this time.

My experience was rewarding and enriching because of many persons. I am grateful to Col. John H. Cissik for accepting my assignment to Wilford Hall Medical Center. Dr. Douglas W. Marshall provided support, direction, and an environment in which I could grow. Dr. Gary S. Gronseth permitted me to learn as I worked on his study. These two capable mentors are also the most gentle teachers a student can ever have.

I am indebted to extraordinarily supportive staff whose help was invaluable in overcoming many technical roadblocks: Dr. D. Fiducia and Mr. Doug Leger, Department of Psychiatry; Ms. Joni Pounds, Department of Neurology; Mr. A. J. Nowatny, HIV Data Research Center; Ms. Terri Rodriguez, Patient Records; and dedicated staff in the Medical Library who fulfilled my research requests. Thanks too to Hospital Volunteers-In-Green who directed me down the right hall whenever I made a wrong turn. And eight hundred AIDS patients earned my admiration and deserve my thanks for allowing their life stories to shine a light towards cure and hope.

I. INTRODUCTION:

It is only since about 1986 that medical doctors, neurologists, psychiatrists, and psychologists have noted presumed association between cognitive deficits, motor deficits, and mood- or depression disorders and infection with acquired immunodeficiency virus (AIDS). These early researchers responded to the striking symptomatology they saw but actually they had rather small numbers of cases of AIDS dementia and the early sample of cases was perhaps biased towards the more symptomatic cases. It had been believed that clinical symptoms of AIDS dementia occur in as many as sixty per cent or more of AIDS cases and that AIDS dementia symptoms could occur even before clinical symptoms became apparent. These early researchers had little opportunity to study significantly large groups of AIDS patients.

My personal review of articles published on AIDS dementia through spring 1988 led me to believe these rather grim statistics and also led me to believe in the presumed association between the so-called neurotrophic affinity of AIDS virus often to infect the human brain early in a patient's AIDS infection.

The Departments of Neurology, Medicine, and the Human Immunodeficiency Virus (HIV) Unit at the USAF Medical Center, Wilford Hall, Lackland Air Borce Base, Tx. are studying a data base of over eight hundred persons seropositive for HIV on a wide range of medical, neurological, and neuropsychological (cognitive) factors. This large-scale longitudinal study seeks to clarify and identify possible physiological predictors and trends in the clinical and neuropsychological status of patients seropositive for AIDS virus. Hopefully, this may lead to increased knowledge, more accurate prognosis, and earlier inter-

intervention and treatment.

My interest in neuropsychology and my familiarity with some of the earlier professional literature on AIDS dementia contributed to my assignment at the Department of Neurology.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Our initial research objective was to prepare draft manuscripts of two sub-studies from among the eight hundred patients seropositive for HIV This was to be preceded by a brief but careful doublecheck for correct match between patient name, subject study number, and computer keying of parameters to be analysed; a seemingly straightforward task that can get confounded when two patients have the same name, when two cases have the same social security number-suffix (patients related to eachother), or when identifying data is coded in complementary though not identical lists for purposes of patient confidentiality.

The first initial sub-study of 124 cases was examining spinal fluid immunoglobulin values in patients seropositive for HIV but who were otherwise asymptomatic. Marshall and Brey et al (1988) and Goethe and Mitchell et al (1989) had previously found no significant neuropsychological differences as a function of cerebrospinal fluid abnormalities in asymptomatic HIV-infected patients.

The second sub-study of about 98 cases not overlapping with the 124 cases was reexamining the 98 cases at intervals of approximately one year to track scores on a battery of neuropsychological tests for verbal fluency,

visuo-motor speed, coordination, mental tracking, finger tapping dexterity, auditory processing, visual memory, and short- and longer-term auditory memory and processing. Data from second evaluations and in some cases from a third evaluation (although different forms of the same test regimen) was entered into the computer data bank for analysis along with updated clinical data on the progress of these patients.

The initial research objective was altered when we decided to try to obtain a test measure of intellectual and neuropsychological ability which predated onset of HIV infection for the 98 cases. We initiated a process to request computer-to-computer exchange of our patients' scores on the Armed Services Vocational Aptitude Battery which test battery had been presented during an Airman's first week in the military. These scores arrived after the period of my summer research fellowship. We had also tried to obtain our patients' scores on a tested measure of psychological depression-or well-being and general psychological adjustment-as-recalled at first week in the military. It turned out that these scores were unavailable to us except at prohibitive cost.

While awaiting the above test data, we commenced an intense re-compilation and search of medical files for exact dates as to when opportunistic infections had first occurred among a third sub-study of 88 cases. The sub-study was later expanded to 116 cases. I spent the majority of my time on this study under supervision of Dr. Gary Gronseth. Dr. Gronseth was seeking evidence that the Life Table of AIDS patients seen as a group at one point in time would reveal more delayed date of onset and lower frequency of onset of AIDS dementia than previously thought.

III.

a. USAF personnel seropositive for HIV usually return to Wilford Hall Medical Center for medical stabilization and for baseline data and evaluation. They then either remain for further treatment if with active HIV related process or return to a duty station. Patients return to Wilford Hall Medical Center for reevaluation approximately once yearly and more often if interim medical treatment is necessary. About eight hundred seropositive patients have been evaluated to date.

The first initial sub-study was to examine spinal fluid immunoglobulin values of 124 HIV infected patients but otherwise asymptomatic. These values had already been obtained via lumbar puncture. I collated files of neurological examinations and read entries for clinical exam, electroencephalogram exam, and nerve conduction velocity exam. Each patient was noted as clearly normal or abnormal and the data was entered onto a study list preparatory to data entry. Data files that were equivocal or unclear were set aside for decision to be made by Dr. Marshall.

b. Data results have not yet been analysed.

IV.

a. The second sub-study re-examined test scores on a battery of neuropsychological tests and compared possible significant differences in scores obtained from re-tests with the original test scores. Changes would be analysed in tandem with any changes in cerebrospinal fluid parameters such as numbers of immunoglobulins (markers for presence of B-cell subtypes), T - helper cell numbers and ratios of T-helper cell subtypes, and the Walter-Reed classification of staging of severity of HIV infection.

Two years ago the sub-study had contained about 78 cases. Now there were 98 cases not overlapping with the first sub-study of immunoglobulin values in 124 cases HIV seropositive but otherwise asymptomatic

I observed the neuropsychology technician administer the test battery. I was oriented as to the purpose and standard responses for each subtest. Our two-person team entered test battery data onto response sheets coded for computer input. Some patients had received a second re-evaluation for a total of three year's data at one-year intervals although the test battery had been altered slightly in the last year. Scores for these latter patients could provide a hint in data trend .

We commenced formal requests through military channels to obtain and match our patients' neuropsychological test battery scores with the closest approximation to pre-HIV infection level of functioning viz. Armed Services Vocational Aptitude Battery scores which also provides a measure of estimated cognitive and intellectual skills. I obtained manuals and literature on this prior test and located a military specialist who advised as to the types of score components we should request. This military psychometrician will be available to the researchers to assist in data interpretation and analysis.

Efforts to obtain test scores for our patients on a military-administered questionnaire of pre-military social adjustment and mood-tendency were made.

Clinical psychiatric data and psychiatric test data previously available for the 98 cases was found to be unentered into the data bank for the past twelve months. This was traced as an oversight and communication gap due to professional staff changes. This data will be entered into the sub-study soon.

b. Data trends for the 78 cases originally re-evaluated was presented by Dr. Marshall at a recent convention Poster Session. Preliminary findings at that time were that cases,which might have progressed toward more clinical severity in terms of Walter-Reed stage,did not seem to re-test with increased cognitive deficit. In fact, neuropsychological test battery scores tended to improve from the previous year levels. The preliminary interpretation of the unexpected finding in the 78 cases originally re-evaluated was that poor test performance at that time was attributed to heightened anxiety, depressed mood, and impaired ability to concentrate or remember during the period when a patient has first learned he is infected with HIV . This is why measures of depression and adjustment were added to the later test battery and why scores on the military-administered questionnaire of pre-military social adjustment and mood-tendency (the HOI Test) were sought. Unfortunately, the HOI Test data had been stored in another computer system in an inappropriate format and the cost to obtain this data manually is prohibitive. Scores for the entire 98 cases in the sub-study will now be matched for Walter-Reed staging, cerebrospinal fluid values, and Armed Services Vocational Aptitude Battery. Data has not yet been analysed.

V.

a. A complete study file was compiled for each of the 116 cases in the Life Table sub-study. Each file consisted at least of narrative summaries of in-patient evaluations and hospitalizations sufficient to unequivocally trace dates, longitudinal course of symptoms, date of first AIDS seropositivity, date of first Walter-Reed staging, date Center For Disease Control definition/criteria for HIV, most recent Walter-Reed staging and date, date of most recent evalu-

ation at Wilford Hall Medical Center, most recent score on Mini Mental Status exam, date of earliest documented occurrence of opportunistic infection (such as pneumocystis pneumonia, oral hairy leukoplakia, Kaposi's sarcoma), and date, cause-of-death, and autopsy finding for deceased patients. Although much of this data was presumably already available from the Human Immunodeficiency Virus computer data bank, all staging and dates were re-checked by careful re-reading of narrative summaries and entire files. If available, manual scoring of the original Mini Mental Status exam was re-checked. Xeroxes of death certificates were checked and autopsy reports were obtained when possible. Most important was the locating and painstaking review of longitudinal serial narrative summaries in order to accurately obtain the earliest date at which opportunistic infection first occurred in a case. For example,human immunodeficiency virus"wasting syndrome"can be identified ex post facto from review of later narrative summaries but is apt to be missed in a forward-only study of narratives. Corrections were made to the Human Immunodeficiency Virus computer data as necessary. Data has not yet been analysed.

b. Professional literature to date tends to consider that symptoms of AIDS dementia occur much earlier and much more commonly in persons with AIDS than would seem to be suggested by gross analysis of Life Table data obtained in this sub-study. There are two sets of Life Table data for 87 of the 116 cases. Stroke tally review of these 87 cases disclosed changes in important Life Table dates for 57 of the 87 cases namely the date of earliest documented occurrence of opportunistic infection was set back (rather than advanced) the following amounts of months earlier than previously known: set back by 1 month for each of 16 cases; 2 months for each

of 6 cases; 3 months for each of 7 cases; 4 months for each of 2 cases; 5 months for each of 2 cases; 6 months for each of 4 cases; 7 months for each of 2 cases; eight months for each of 1 case; 9 months for each of 2 cases; 10 months for each of 2 cases; 11 months for 1 case; 12 months for each of 3 cases; 13 months for 1 case; 14 months for 1 case; 15 months for 1 case; 16 months for each or 4 cases; 23 months for 1 case; 36 months for 1 case; 49 months for 1 case. In 3 cases the narrative information was still unclear to me. Chart missing for 1 case. The data must now be analysed to determine how these changes may affect the original Life Table calculation. For example, does 16 cases with a one-month change counterbalance 1 case with a 36-month change? How soon and with what frequency does AIDS dementia occur in the statistical patient's course-of-AIDS?

It became necessary to operationally define the "date of earliest documented occurrence of opportunistic infection. I set this perhaps arbitrarily although applied uniformly in all 116 cases as "date symptom first appeared chronologically in narrative summary." This is to be distinguished from date a symptom was first diagnosed. It is also to be distinguished from date a narrative-first-describing-a-symptom is dictated or typed. It is by this convention that the greatest frequency of changed (reduced) dates occurred. This must be noted when re-calculating the Life Table.

It is also helpful to remember that AIDS dementia is diagnosable only when other possible diagnoses are excluded. Neurosyphilis, toxoplasmosis, cryptococcol meningitis, and cytomegalovirus infection of brain all may cause neurological symptoms in a patient who has succumbed to these infections while immunocompromised by AIDS but these are not synonymous with AIDS dementia. True AIDS dementia as described by Price and Brew et al (1988)

a variable, yet characteristic, constellation of abnormalities in cognitive, motor, and behavioral function ... salient are the slowing and loss of precision in both mentation and motor control... complex but formerly routine mental tasks take longer and need to be consciously broken down into component steps. Concomitantly, these patients often lose interest in their work as well as in their social and recreational activities. It is this growing apathy along with mental slowing that is frequently mistaken for depression....Motor symptoms usually lag somewhat behind,but exaggerated tremor or mild gait unsteadiness may be among early complaints, while on examination, slowing of rapid alternating eye and extremity movements and abnormal 'release' reflexes are common.

.....Increasing apathy, slowing of speech, and mental impoverishment ultimately may progress to near or absolute mutism and severe dementia...in some patients an agitated mental state with mania or other forms of organic psychosis may occur; in others, weakness with progressive paraparesis dominates the course.

The Life Table sub-study finds up to 5 cases of AIDS dementia among the 116 cases. The exact data now compiled remains to be analysed.

VI. RECOMMENDATIONS:

a. In my opinion it would be advantageous to safeguard and enhance with new logistical procedures the invaluable, large, and unique collection of data on U.S. Air Force personnel seropositive for AIDS virus. This patient group is invaluable because of its size (over 800 cases) and because patients do not self-select or volunteer for study or treatment but rather

more typically are discovered upon routine military health screening. It is a large sample. It is heavily biased towards active, healthy military volunteers. Much valuable research and treatment interventions can be initiated with such a study group; certain logistics and procedural policies will enhance and speed such research:

Many military AIDS patients may choose to receive evaluation and care at Wilford Hall Medical Center. If these patients retire from the Air Force they may revert to Veterans Administration or private sector health care providers. This represents an incalculable loss of continuity of data for research and it permits less than optimal continuity of patient care. AIDS patients should be strongly recruited to return to Wilford Hall Medical Center for continuing treatment and evaluation. Patients who obtain treatment in Veterans Administration hospitals should have case data automatically reported back to Wilford Hall Medical Center. Additionally and in particular, autopsy data and date-and-cause-of-death information should be returned to Wilford Hall Medical Center in a routine procedure. Technical staff assistance should be budgeted if necessary to track such information;

AIDS patients'medical files should be tagged with electronic sensors so they cannot be removed from the hospital building. AIDS patient medical files should not be permitted to leave the hospital building for long-term storage in St. Louis or certainly not unless they have been completely micro-film-copied first. The medical records file rooms should be organized so that it is not onerous to search and refile accurately. For AIDS patients' medical files a distinct color/style binder should be considered. The in-patient computer registry system should contain registration numbers for

all in-patient hospitalizations--the current system may drop from memory the data from in-patient stays prior to 1983 without informing the searcher about the pre-existing files. Sufficient staff or volunteers should be assigned to retrieve and re-file medical records so that researchers are not needlessly delayed and do not have to pull their own files. A Master Log of AIDS file charge-outs should be maintained at the in-patient record room;

Centralized responsibility for overseeing AIDS research and treatment should also imply centralized sharing of information. A Technical Assistance Coordinator should maintain and disseminate updated lists of which departments are collecting which data for what studies, how other researchers may share or add to this data, access it, or peruse it. A Technical Assistance Coordinator should facilitate the sharing of data collected in experimentors' files and among the in-patient and between in-patient and out-patient services. That is, to the greatest extent possible and within guidelines of patient confidentiality, research and treatment data collected should be available and known to fellow reseearchers viz. duplication of effort is to be avoided. The Technical Assistance Coordinator position should be budgeted if necessary;

The centralized body that directs AIDS research and treatment in Wilford Hall Medical Center should design and define its expectations for its own members as well as its expectations and responsibilities for inter-hospital and inter-military exchange of information;

Procedural-technical improvements may include: re-programming medical transcription machines to include patient's date of birth on all pages of all patient narrative summaries. Patient's date of birth should also ap-

pear as a matter of course on all pages of all autopsy reports. Wilford Hall Medical Center Telephone Directory and Lackland Air Force Base Telephone Directory should contain an alphabetical listing of facilities and services in everyday words such as the layman might use when searching for a listing e.g. Pediatrics; Child; Baby; or Brain; Haad; Nerves; Neurology.

b. Certain of the Life Table sub-study of 116 cases should be re-examined from viewpoint of epidemiology or infection transmission of pneumocystis carnii pneumonia among patients who appear to be free of this infection upon entry to the AIDS service. I believe 2 or more patients developed new pneumocystis carnii pneumonia just prior to dismissal from Wilford Hall evaluation terms. I suggest a quick re-review of narrative summaries for at least the following study cases: 43,33, 496, 292, 190, 349, 137, 875, 361, 548, 56, 186, 117, 225, 174, 81, 29, 41, 349, 631.

c. The data entry coding guidelines to the AIDS patient data base can be revised to accomplish by machine the entry into "earliest documented occurrence of opportunistic infection" which I recorded by hand: Center For Disease Control defines four topics (computer columns) or classifications of surveillance criteria which may be used to assign or define AIDS; the medical personnel who assign Walter-Reed staging or medical records coding to the medical chart can code for input the date and exact opportunistic infection at the same time.

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d. Preliminary reports of autologous blood transfusion from AIDS seropositive patients at low Walter-Reed staging (less immunocompromised) back to self-as-recipient (after treating the blood to kill viral components) appears a possibly beneficial treatment procedure that slows or reverses the course of clinical symptoms. In telephone conversation with researchers (Bruster, Kuntz, et al 1988), it was noted that the large number of HIV seropositive patients asymptomatic or at low Walter-Reed staging is an ideal sample in which to undertake large-scale test of autologous blood transfusion treatment. Maximization of benefits from early high T-helper count in self-host would appear to reinforce preliminary cerebrospinal immunological hypotheses being formulated now at Department of Neurology, Wilford Hall Medical Center. Autologous blood transfusion study at Wilford Hall Medical Center may be a logical next step in maintaining and advancing clinical research and treatment intervention without excessive cost. A promising treatment intervention can be tested; seropositive personnel can be cycled to active duty, and military research may gain well-deserved acclaim. Autologous transfusion procedures and protocols are being forwarded to Department of Neurology, Wilford Hall Medical Center for perusal. Military staff have been invited to tour the researchers' laboratories and Institute at University of Duesseldorf, W. Germany.

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1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM

GRADUATE STUDENT RESEARCH PROGRAM

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FINAL REPORT

Comparison of Thromboelastography (TEG) versus Standard Hematologic Parameters to Predict Hemmorhage after Cardiopulmonary Bypass (CPB)

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Date :	October 25,1989
Contract No. :	F49620-88-C-0053

Comparison of Thromboelastography (TEG) versus Standard Hematologic Parameters to Predict Hemorrhage after Cardiopulmonary Bypass (CPB)

by

John Y. Salinas

ABSTRACT

Fifteen Patients were studied over a ten week period to study TEG versus more standard hematologic tests in an attempt to determine which tests are more predictive of post-operative bleeding complications. Specifically, cardiopulmonary bypass patients were selected for their increased post-operative bleeding complications and significant morbidity and mortality. Pre-operative and post-operative TEG's, bleeding time, prothrombin time, partial thromboplastin time, hemoglobin, hematocrit, platelet count, fibrinogen, and fibrin split products, activated clotting time, duration of bypass, duration of operation, and mean platelet volume were measured. Specific TEG parameters were also measured including R interval, angle of clot formation, k interval, and maximum amplitude of clot strength formation. Preliminary results indicate a significant difference between pre and post operative TEG parameters in concurrence with standard hematologic parameters.

Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems must be mentioned for their concern and help to me in all administrative and directional aspects of this program. I also thank my effort focal point at Wilford Hall USAF Medical Center, Colonel John Cissik, for his assistance and logistical support and guidance throughout this endeavor.

My experience was particularly rewarding and enriching because of the professional and friendly relationship between myself and the many inspiring members of the Hematology-Oncology Service within the Department of Medicine at Wilford Hall USAF Medical Center, who created a truly enjoyable working atmosphere. Specifically, I would like to express my gratitude to Captain James Essel and Captain Tom Martin, each of whom I had daily contact with, and who served as a source of stimulation and collaboration during this project. Colonel Jim Thompson's interest in every phase of this project , as well as advice and support was also appreciated for the contribution to this project. Finally, I would like to thank all of those who contributed their time and laboratory support during data acquisition, particularly the staff of the Special Hematology Laboratory.

I. INTRODUCTION :

Despite the technical advances made in open heart surgery 2-5% of patients will experience bleeding complications requiring postoperative re-exploration of the chest. The effect of cardiopulmonary bypass (CPB) on the hemostatic system is complex. Consumption of coagulation factors and platelets during cardiopulmonary bypass is well documented. However these decreases are rarely severe enough to result in clinical bleeding Instead an impairment of platelet function appears to be of greater importance in post cardiopulmonary bypass hemorrhage.

Abnormal platelet function has been demonstrated by obtaining preoperative and postoperative bleeding times. These studies reveal a postoperative prolongation of the bleeding time followed in CPB patients who display clinically normal hemostasis and in those with postoperative hemorrhage. The prolonged bleeding time as well as the hemorrhagic tendency can be reversed with platelet transfusions. However, as platelet transfusions are expensive and carry the risk of transmitting disease, prophylactic platelet transfusions are not indicated.

Currently there are no standard parameters to predict postoperative hemorrhage in those patients with adequate platelet counts and normal prothrombin time (PT) and partial thromboplastin time (PTT). Spiess has studied the use of TEG in cardiopulmonary bypas

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II. OBJECTIVES OF THE RESEARCH EFFORT

This study proposes to corroborate Spiess' data while comparing the TEG data obtained to a more expanded profile of hematologic tests including bleeding time, platelet size and von Willebrand Factor level. We hope to determine specific parameters that will predict those patients at highest risk for post-operative hemorrhage. Future trials could then be developed where those patients at highest risk could be treated prophylactically with DDAVP and/or platelets to determine if bleeding complications can be reduced, while at the same time limiting unnecessary transfusions. Although this study has managed to study fifteen of the proposed forty patients, it will be valuable in establishing trends and modifications necessary in the acquisition of statistical data required for analysis of this ongoing research at Wilford Hall USAF Medical Center. The data acquired will be studied with statistical programs incorporating T-tests of the two groups, pre and post-operative, isolating statistically significant parameters.Pearson Correlation coefficients using bleeding time compared to the other data collected will also be studied to assess the feasability of ordering particular laboratory tests in patients with bleeding diathesis. This will also incorporate the TEG data, both pre and post-operatively.

III. TECHNICAL APPROACH

In this particular study, fifteen of the planned forty patients undergoing cardiopulmonary bypass surgery were studied. Eligibility for participation required that the patients be greater than eighteen years and that signed informed consent was enacted. Samples required included pre-operative, within 24 hours prior to surgery, and post-operative, upon arrival in the intensive care unit TEG's requiring approximately 0.70 cc of whole blood drawn through an arterial line. Pre and post-operative bleeding times were also obtained, along with thrombin time, prothrombin time, partial thromboplastin time, fibrinogen, fibrin split products, factor VII C, factor VIII, and complete blood count, requiring in total about 45cc of whole blood. All samples except bleeding time were drawn via arterial catheter placed for surgery. TEG was performed in the OR as currently performed as standard of care. All tests were run in the Special Hematology Lab. Progress charts which included intraoperative data and post-operative data monitoring the patients clinical course were maintained throughout the study of all patients. This included length of bypass, operation, amount of chest tube drainage, input and output, type of preoperative anti-coagulation, estimated blood loss, amount of blood products administered, DDAVP, EACA, and fresh frozen plasma.

NUMBER OF VALID OBSERVATIONS (LISTWISE) = 9.00

PRE

VARIABLE	MEAN	STD DEV	MINIMUM	MAXIMUM	VALID N	LABEL
[illegible]	1.364	.505	1.00	2.00	11	
[illegible]	5.318	1.47[illegible]	2.50	8.00	11	
[illegible]	12.009	1.[illegible]	9.50	15.30	11	
[illegible]	35.5[illegible]	5.[illegible]	27.90	45.90	11	
[illegible]	315.45[illegible]	99.19[illegible]	136.00	415.00	11	
[illegible]	8.609	.[illegible]	7.60	10.90	11	
[illegible]	12.240	.[illegible]	10.10	19.00	10	
[illegible]	12.255	.[illegible]	11.80	14.20	11	
[illegible]	32.500	11.[illegible]	24.40	66.40	11	
[illegible]	163.63[illegible]	[illegible]	[illegible]	365.00	11	
[illegible]	131.455	[illegible]	113.00	203.00	11	
[illegible]	15.659	[illegible]	9.[illegible]	41.00	11	
[illegible]	5.909	.[illegible]	3.00	11.50	11	
[illegible]	41.[illegible]	11.[illegible]	26.00	60.00	11	
[illegible]	62.[illegible]	10.[illegible]	43.00	83.00	11	
[illegible]	60.[illegible]	10.[illegible]	49.00	83.00	10	
[illegible]	11.[illegible]	[illegible]	3.50	23.00	11	
[illegible]	9.409	1.[illegible]	7.00	11.90	11	
[illegible]	27.791	4.[illegible]	20.00	36.00	11	
[illegible]	110.7[illegible]	47.[illegible]	43.00	190.00	11	
[illegible]	8.[illegible]	.[illegible]	6.90	9.40	11	
[illegible]	1.[illegible]	[illegible]	7.30	16.30	11	
[illegible]	17.[illegible]	1.[illegible]	13.50	20.70	11	
[illegible]	40.[illegible]	[illegible]	28.[illegible]	60.70	11	
[illegible]	12[illegible]	[illegible]	77.[illegible]	177.00	11	
[illegible]	1.[illegible]	.[illegible]	90.00	175.00	11	
[illegible]	11.318	.[illegible]	7.00	15.50	11	
[illegible]	5.455	4.[illegible]	2.50	18.00	11	
[illegible]	47.545	15.[illegible]	14.00	51.00	11	
[illegible]	49.409	[illegible]	5.00	66.00	11	
[illegible]	42.455	[illegible]	27.00	58.00	11	
[illegible]	5.[illegible]	1.[illegible]	4.00	8.25	11	
[illegible]	2.[illegible]	1.[illegible]	1.[illegible]	6.16	11	

POST

Page

VMS VS.. PRE

NONE SIGNIFICANT

T - T E S T

GROUP 1 [illegible] 1.00
GROUP 2 [illegible] 2.00

VARIABLE		NUMBER OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	F VALUE	2-TAIL PROB.	POOLED VARIANCE ESTIMATE T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.	SEPARATE VARIANCE ESTIMATE T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.
BLEED	GROUP 1 (NON BLEEDER)	[illegible]	5.[illegible]	[illegible]	0.36[illegible]	6.80	0.047	0.85	[illegible]	0.41[illegible]	[illegible]	3.31	0.54[illegible]
	GROUP 2 (BLEEDER)	1	5.[illegible]	[illegible]	1.[illegible]48								
HGB	GROUP 1	[illegible]	[illegible]	[illegible]	0.641	1..6	0.910	1.51	[illegible]	[illegible]	1.5[illegible]	[illegible].05	0.16[illegible]
	GROUP 2	4	11.[illegible]	[illegible]	0.[illegible]54								
HCT	GROUP 1	[illegible]	37.[illegible]	[illegible]	1.956	1.18	0.969	1.5[illegible]	[illegible]	[illegible]	1.5[illegible]	6.05	0.16[illegible]
	GROUP 2	4	32.4[illegible]	[illegible]	[illegible].385								
PLT	GROUP 1	[illegible]	[illegible]35.[illegible]	[illegible]	34.[illegible]	7.50	0.1[illegible]7	1.14	[illegible]	[illegible]	1.4[illegible]	[illegible].[illegible]3	0.188
	GROUP 2	4	180.0000	[illegible]	16.097								
[illegible]	GROUP 1	[illegible]	[illegible]	[illegible]	0.178	9.[illegible]	0.023	-1.6[illegible]	[illegible]	0.1[illegible]	1.3[illegible]	3.38	0.27[illegible]
	GROUP 2	4	9.[illegible]	[illegible]	0.717								
PT	GROUP 1	[illegible]	[illegible]	[illegible]	0.[illegible]04	9.87	0.025	-1.44	[illegible]	0.1[illegible]	0.[illegible]	[illegible].18	0.431
	GROUP 2	[illegible]	14.[illegible]	[illegible]	.419								
PTT	GROUP 1	[illegible]	[illegible]	[illegible]	0.160	6.96	0.044	-0.58	[illegible]	[illegible]	0.4[illegible]	3.70	0.672
	GROUP 2	4	12.[illegible]	1.1[illegible]	0.559								

- T - T E S T -

GROUP 1 - GROUP EQ 1.00
GROUP 2 - GROUP EQ 2.00

								POOLED VARIANCE ESTIMATE			SEPARATE VARIANCE ESTIMATE		
VARIABLE		NUMBER OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	F VALUE	2-TAIL PROB.	T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.	T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.
[illegible]	GROUP 1	7	28.7000	3.259	1.232	31.20	0.001	-1.54	9	0.158	-1.14	3.11	0.335
	GROUP 2	4	39.1500	18.204	9.102								
[illegible]	GROUP 1	7	261.8571	[illegible]	27.335	16.33	0.043	-0.13	9	0.899	-0.1?	7.19	0.870
	GROUP 2	4	266.7500	17.896	8.948								
[illegible]	GROUP 1	[illegible]	125.14??	10.463	3.955	15.41	0.006	-1.10	9	0.300	0.83?	3.22	0.464
	GROUP 2	4	142.5000	41.073	20.537								
R	GROUP 1	7	12.9643	?.671	1.010	26.99	0.001	-1.42?	9	0.188	-1.06?	3.13	0.365
	GROUP 2	4	20.3750	13.877	6.938								
K	GROUP 1	[illegible]	5.3571	?.704	1.022	1.35	0.686	-0.85	9	0.419	-0.81	5.57	0.452
	GROUP 2	4	6.0750	3.146	1.573								
G	GROUP 1	7	43.8571	13.42?	5.073	2.35	0.516	0.51	9	0.62?	0.58	8.66	0.580
	GROUP 2	4	40.0000	8.756	4.378								
NA	GROUP 1	7	60.5714	6.214	2.349	7.47	0.038	-0.64	9	0.538	-0.50	3.47	0.646
	GROUP 2	4	65.0000	16.990	8.495								
[illegible]	GROUP 1	7	57.0000	6.3?5	2.390	5.36	0.092	-2.01	8	0.080	-1.44	2.33	0.269
	GROUP 2	[illegible]	69.6667	14.640	8.452								

Marginal

[illegible] WILFORD HALL MEDICAL CENTER [illegible]

POST for All tegs two groups sig. different

GROUP 1 [illegible] EQ 1.00
GROUP 2 [illegible] EQ 2.00

VARIABLE		NUMBER OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	F VALUE	2-TAIL PROB.	POOLED VARIANCE ESTIMATE T VALUE	POOLED VARIANCE ESTIMATE DEGREES OF FREEDOM	POOLED VARIANCE ESTIMATE 2-TAIL PROB.	SEPARATE VARIANCE ESTIMATE T VALUE	SEPARATE VARIANCE ESTIMATE DEGREES OF FREEDOM	SEPARATE VARIANCE ESTIMATE 2-TAIL PROB.
BLEED	NONBLEEDER GROUP 1	7	?.5714	4.??	1.??	2.??	0.364	1.07	[illegible]	0.314	0.95	4.57	0.3??
	BLEEDER GROUP 2	4	1?.5000	2.41?	.70?								
HGB	GROUP 1	[illegible]	?.9429	[illegible]	0.???	17.00	0.041	1.?5	[illegible]	0.209	1.77	7.15	0.11?
	GROUP 2	4	10.2250	0.44?	0.22?								
HCT	GROUP 1	[illegible]	26.5000	[illegible]	2.175	12.14	0.066	1.17	[illegible]	0.272	1.51	7.54	0.1??
	GROUP 2	4	?.0500	1.??	0.???								
FIB	GROUP 1	[illegible]	1??.2857	[illegible]	15.??	1.14	0.809	1.??	[illegible]	0.0??	1.84	?.00	0.11?
	GROUP 2	4	??.2500	[illegible]	2?.???								
MPV	GROUP 1	[illegible]	?.3286	0.??4	0.?40	2.?4	0.288	0.61	[illegible]	0.554	0.53	4.34	0.6??
	GROUP 2	4	?.0250	1.031	0.515								
PT	GROUP 1	7	1?.9714	[illegible]	1.12?	1.31	0.711	0.56	[illegible]	0.589	0.54	5.76	0.611
	GROUP 2	4	11.8750	[illegible]	1.7??								
PTT	GROUP 1	7	17.9000	1.400	0.5??	4.44	0.115	0.??	[illegible]	0.7??	0.22	[illegible]	0.8??
	GROUP 2	4	17.2500	[illegible]	1.175								

- - - - - T - T E S T - - - - -

GROUP 1 - GROUP EU 1.00
GROUP 2 - GROUP FU 2.00

VARIABLE		NUMBER OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	F VALUE	2-TAIL PROB.	POOLED VARIANCE ESTIMATE T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.	SEPARATE VARIANCE ESTIMATE T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.
PIL	GROUP 1	7	37.8286	5.925	3.373	2.15	0.389	-1.15	9	0.281	-1.02	4.64	0.35[illegible]
	GROUP 2	4	45.3750	13.100	6.550								
FIB	GROUP 1	7	128.7143	44.[illegible]	16.740	1.80	0.673	0.[illegible]	9	0.777	0.32	8.0[illegible]	0.7[illegible]
	GROUP 2	4	121.2500	[illegible]	16.494								
[illegible]	GROUP 1	[illegible]	1[illegible].5714	2[illegible].47[illegible]	9.372	2.11	0.578	0.6[illegible]	9	0.52[illegible]	0.73	8.45	0.4[illegible]
	GROUP 2	4	123.7500	1[illegible].174	8.0[illegible]7								
(1)	GROUP 1	7	10.3571	1.[illegible]	0.[illegible]	1.1[illegible]	0.820	2.46	[illegible]	0.03[illegible]	2.42	[illegible].04	0.0[illegible]
	GROUP 2	4	13.0000	1.[illegible]	0.[illegible]								
(1.)	GROUP 1	7	3.2857	0.4[illegible]	0.184	168.35	0.000	[illegible]	9	0.0[illegible]	1.88	3.02	0.1[illegible]
	GROUP 2	4	9.2500	6.331	3.166								
(G2)	GROUP 1	7	56.0000	4.000	1.512	18.06	0.004	3.59	9	0.006	2.69	3.1[illegible]	0.0[illegible]
	GROUP 2	4	32.7500	16.[illegible]	8.4[illegible]								
(MA2)	GROUP 1	7	55.3571	5.7[illegible]	2.168	1.76	0.507	4.06	9	0.003	3.73	[illegible].00	0.014
	GROUP 2	4	[illegible].0000	7.616	3.803								
(A[illegible])	GROUP 1	7	47.8571	8.668	3.276	4.03	0.280	3.16	9	0.012	3.79	8.96	0.004
	GROUP 2	4	33.0000	4.[illegible]	2.160								
[illegible]	GROUP 1	7	6.0686	1.974	0.746	2.72	0.441	0.9[illegible]	9	0.384	1.05	8.07	0.3[illegible]
	GROUP 2	4	5.0625	1.197	0.5[illegible]								
HYLA	GROUP 1	7	3.2129	1.397	0.528	1.14	0.983	1.27	9	0.235	1.30	6.7[illegible]	0.2[illegible]
	GROUP 2	4	2.1250	1.29	0.650								

correlation coefficients for pre parameters and pre. bleeding time

Three, Act, K + G are significantly larger than zero.

- - PEARSON CORRELATION COEFFICIENTS - -

	HGB	HCT	PLT	MPV	TT	PT	PTT	FIB	ACT	R	K
BLEED TIME	[illegible]	.2255	[illegible]	.5485	.1395	.4859	.5461	.2380	.6[illegible]	[illegible]	.6106
	(11)	(11)	(11)	(11)	(10)	(11)	(11)	(11)	(11)	(11)	(11)
	P= [illegible]	P= .50[illegible]	P= [illegible]	P= .081	P= .701	P= .130	P= .082	P= .479	P= [illegible]	P= [illegible]	P= .046

	G	MA	ANG
BLEED TIME	.62[illegible]	.5089	.1467
	(11)	(11)	(10)
	P= .038	P= .110	P= .685

Pearson r's for post bleeding time none are sig. larger than zero

- - PEARSON CORRELATION COEFFICIENTS - -

	HGB2	HCT2	PLT2	MPV2	TT2	PT2	PTT2	FIB2	ACT2	R2	K2
BLEED TIME POST	.01[illegible]	.0281	.4729	.0486	-.0353	.3744	.2780	-.1689	.3205	.4[illegible]43	.2640
	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)	(11)
	P= .971	P= .935	P= .1[illegible]	P= .887	P= .918	P= .257	P= .408	P= .620	P= .337	P= .160	P= .433

(COEFFICIENT / (CASES) / 2-TAILED SIG) " . " IS PRINTED IF A COEFFICIENT CANNOT BE COMPUTED

	G2	MA2	ANG2
BLEED TIME POST	.322[illegible]	.2560	.2598
	(11)	(11)	(11)
	P= .33[illegible]	P= .447	P= .440

(COEFFICIENT / (CASES) / 2-TAILED SIG) " . " IS PRINTED IF A COEFFICIENT CANNOT BE COMPUTED

IV. DISCUSSION OF DATA

In the analysis of the data retrieved and tabulated, multiple t-tests were done on various components to specifically determine trends and future approaches to retrieval and analysis once all the appropriate patients were completed in the project. In particular, the fifteen patients were broken down into bleeders and nonbleeder experiencing bleeding diathesis post-operatively. Bleeders were defined as those patients having greater than 1500cc of chest tube drainage or requirement for platelet transfusion. Also four patients were identified as having grossly abnormal TEG tracings. This was determined by at least two TEG parameters deviating from the expected values. Only three patients required platelets. In addition eight patients required packed red blood cells as determined by the surgeon. None of the TEGs were affected by this supplementation since TEG samples were drawn prior to transfusions.

Specific analysis of data show that T-tests of pre-operative components between bleeders and non-bleeders showed only a marginal significance in the A60 parameter of the TEG tarcing, the strength of the clot 60 minutes after the maximum amplitude of clot formation. Otherwise there were none significant parameters showing discrepancy between

bleeder and non-bleeders. This included all laboratory values, as well as TEG parameters obtained from study of the TEG tracings. None were significant. In contrast, study of T-test of post-operative data between bleeders and non-bleeders showed significant discrepancy between four specific parameters. The bleeders varied significantly from the non-bleeders in TEG parameters such as R interval K interval, angle of clot formation, and maximum amplitude width of the TEG tracing. Also as in the pre-operative data A 60 data displayed a discrepancy in the bleeders vs. non-bleeders. The significance was less than 0.050 in all parameters significant.

Pearson correlation coefficients were also done as part of the statistical package analyzing the data. Pre-operative data was studied for correlations between bleeding time and all the other data collected. Significant correlations were found between bleeding time and ACT (activated clotting time), and angle of clot formation, as well as K interval. The significance was less than 0.050 in each. Bleeding time was selected because it provided a means and method to monitor platelet function, which has a direct effect on coagulation and bleeding diathesis. Pearson correlation coefficients of post-operative data correlating bleeding time with all the other laboratory and TEG data found significant correlations in none of the parameters. All

the p confidence intervals were much greater than 0.050 with the closeset significant parameter being R interval with a confidence interval of 0.160 .

Thus, in both pre-operative and post-operative data collected and analysed, the TEG (thromboelastography) parameters showed a better correlation with bleeding time than any of the other laboratory data except ACT . This is encouraging information in support of the use of TEG thromboelastography in the overall determination of assessing bleeding diathesis of post-operative patients possibly requiring transfusions of coagulation components. Particular to mention in the context of this discussion is the fact that bleeding times pre-operatively and post-operative means and standard deviations were significantly different demonstrating the need of assessing platelet function in the coagulation process. Specifically, bleeding time pre-operatively was 5.318 mean, with a standard deviation of 1.617, and post-operatively was 11.0 with a standard deviation of 5.920. Furthermore in the initial analysis of t-tests, it was once again the TEG parameters which were found to be significantly different between bleeders and non-bleeders, a fact also demonstrated in the Pearson correlation coefficient analysis. This was a finding that was not consistently duplicated by the other hematologic tests. This encouraging finding, if maintained throughout the

completion of the study of all forty patients, would be an encouraging sign for the possible use of TEG , thromboelastography, as a standard of care in the assessment of post-operative screening and treatment indications of patients with bleeding diathesis.

V. RECOMMENDATIONS

In the initial analysis of this study, it has been demonstrated that TEG, thromboelastography, has a potential use in the assessment and screening of patients with significant bleeding diathesis requiring transfusion. TEG can be useful in predicting patients at highest risk for post-operative hemorrhage, and will make a valuable tool in the arsenal of post and pre-operative test aiding in the clinical care course and assessment of patients. With these hopes, future trial could then be developed where those patients at highest risk could be treated prophylactically with DDAVP and /or platelets to determine if bleeding complications can be reduced, while at the same limiting unnecessary transfusions. The present study that is being completed at Wilford Hall USAF Medical Center will serve as valuable impetus in this validation of TEG thromboelatography's important role in patient care. It is the hope and recommendation of this researcher that the above mentioned facility completing this study receive

future funding to continue this valuable and potentially helpful study. The resources and patient volume , as well as the highly adept clinical personnel at Wilford Hall USAF Medical Center make this an ideal facility to continue this research while providing ample data in the assessment of the value of thromboelastography in screening post-operative bleeding diathesis, and eventual treatment.

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